Journal of Applied Pharmaceutical Science Vol. 2 (9), pp. 010-014, September, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.2902
ISSN 2231-3354

Study of Media Effect toward Antimicrobial, Antiplasmodial, Herbicidal Activities and Toxicity of Extract Produced by *Acropora dibranchiata*-*Associated Moraxella* Sp. *RA15*

Risa Nofiani[1]*, Rizmahardian Ashari Kurniawan[1], Tika Mabella[1] , Puji Ardiningsih[1], Titin Anita Zahara[1] and Sunarto[2]
[1]*Department of Chemistry, Mathematics and Natural Sciences Faculty, Tanjungpura University, Jl. A. Yani, Pontianak, Kalimantan Barat, Indonesia.*
[2]*Department of Fisheries, Agricultural Faculty, The University of Muhamadiyah Pontianak, Jl. A. Yani, Pontianak, Kalimantan Barat, Indonesia.*

ARTICLE INFO

Article history:
Received on: 12/3/2012
Revised on: 13/3/2123
Accepted on:23/23/2222
Available online: 28/09/2012

Key words*: Moraxella sp.* RA15*, antimicrobial, herbicidal, antiplasmodium, toxicity.*

ABSTRACT

Marine invertebrate -associated bacteria are one of potential and prolific sources to produce unique and novel structure of bioactive compounds. Unsuitable growth media for cultivation of bacteria result in many bioactive compounds were not produced. *Moraxella* sp. RA15 was successfully isolated from *Acropora dibranchiata*. In an attempt to introduce bioactive compounds of *Moraxella* sp. RA15 was evaluated through various media and bioassays to find out the antimicrobial, antiplasmodial, and herbicidal potency as well as toxicity property. The *Moraxella* sp. RA15 extract in 1/10ZoBell agar medium showed herbicidal activity, toxicity against brine shrimp and antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The antiplasmodial activity was only showed by *Moraxella* sp. RA15 extract produced in 1/10ZoBell broth medium. It can be concluded that medium and culture condition are important factors in bioactive compound production from bacteria.

INTRODUCTION

Marine bacteria have exhibited as a potential source of bioactive compounds such as antimicrobial, anticancer, antiplasmodial, antiviral, antitumor, anticoagulant and cardioactive properties. Compared with terrestrial bacteria, the compound structure produced marine bacteria have more novel and unique structures due to generally halogenated structures with bromine and or chlorine (Gudbjarnason, 1999), high diversity of species and unique living circumstances. In addition, seawater compositions contain various biologically active substance such as vitamin and active agent inhibitory (Okami, 1982). Interactions between marine bacteria and their host organisms are known to play a significant role in produce many bioactive compounds. For instance, bacteria associated with coral demonstrated a higher percentage of bioactive compounds (antibacterial activity) than free living bacteria (seawater) or sediment (Shnit-Orland and Kushmaro 2008). The bioactive compounds may play a role in the coral's defense mechanism (Rohwer *et al.*, 2002). Some of the bioactive compounds show antimicrobial activity such as 20% of cultured bacteria from the mucus layer of the coral *Acropora palmata* displayed antimicrobial activity (Ritchie, 2006). *Pseudomonas* TASC-16 associated with soft coral *Sinularia polydactyla* showed strong growth inhibition against *Streptococcus equi* subsp. zooepidermicus K.6.72 (Radjasa *et al.*, 2007).

* *Corresponding Author*
Tel.: +62561577963. Fax: +62561577963
E-mail: *rnofiani@yahoo.com*

Abundant bacteria community can be found in coral such as the surface mucus layer, coral tissue and the calcium carbonate skeleton (Bourne and Munn, 2005; Koren and Rosenberg, 2006). Potency of bacteria associated with coral as a source of prolific bioactive compounds is promise, but not all bacteria associated coral can produce the bioactive compounds when it is cultivated in the laboratory. There are many factors influencing bioactive compound productions such as types of medium and physical environtment. Therefore, the aim of this study was focused on effect of cultivation *Moraxella* sp. RA15 associated with *A. dibranchiata* in various medium and condition toward biological activities. The extract was tested antimicrobial, herbicidal, antiplasmodial activities and brine shrimp lethality.

MATERIALS AND METHODS

Materials

Moraxella sp. RA15 was successfully isolated from a soft coral which identified as *Acropora dibranchiata*. *A. dibranchiata* was collected in 3-4 m depth from Lemukutan Island, West Kalimantan, Indonesia.

Identification of Isolate RA15

Isolate RA15 was identified based on macroscopic and microscopic examinations as well as biochemical characterization. Microscopic test included gram staining and shape. Biochemical tests included carbohydrate utilization, catalase activity, oxidase activity, aerobic and anaerobic fermented oxidation test of glucose, indole, H_2S, urease, citrate utilization, motility, decarboxylase activity and growth test in TCBS medium. The result of macroscopic, microscopic and biochemical characters were compared to the identification keys.

Screening of Antimicrobial Activity

Microorganism tests were used to evaluate antimicrobial activity of bacterial extract namely *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Eschericia coli*, *Vibrio vara*, *Vibrio cholerae*, *Vibrio harveyi*, *Salmonella* sp. dan *Aeromonas hydrophila* and fungi namely *Candida tropicalis* dan *Candida albicans*.

There were 3 types of medium used to screening of antimicrobial activity namely : 1/10 ZoBell agar (Z^{-1} A), 1/10ZoBell agar supplemented with 1% of glucose (Z^{-1}GA) and 1/10ZoBell agar supplemented with 1% of gliserol (Z^{-1}GLA). 1/10Z broth medium was made with mix 1 part of ZoBell broth medium and 9 part of seawater.

In the preliminary screening of antimicrobial activity was conducted using modified agar streak method. A loopful of *Moraxella* sp. RA15 was streaked circularly onto plate containing screening medium. After incubated at room temperature for 48 hours, plate was inoculated with fresh culture of test microorganism (bacteria and fungi) by a spray then incubated at room temperature for 24 hours. The fresh culture of test bacteria were prepared with inoculated a loopful of colony into 10 mL of nutrient broth then incubated at room temperature 24 h. The fresh culture of test fungi were prepared with a loopful of colony inoculated into 10 mL of potato dextrose broth then incubated at room temperature 24 h. Isolate showing antimicrobial activity was signed with formation of inhibition zone around isolate then diameter of inhibition zone (measured from the edge of the colony to the edge of the clear zone) was recorded.

Inoculum preparation

A loofpull of *Moraxella* sp. RA15 was inoculated onto 10 mL of broth medium and incubated on a rotary shaker incubator at 130 rpm at 30 ^oC for 16 h. Fifty µL of culture suspension was used as an inoculum in cultivation.

Cultivation and Extraction of *Moraxella* sp. RA15 Culture

Moraxella sp. RA15 isolate was inoculated and cultured on different nutrional media namely solid and broth medium. Solid medium used for this study was Z^{-1}A. Broth medium was M13 broth, ZoBell (Z broth), Z^{-1}, and B1 broth.

Cultivation of *Moraxella* sp. RA15 was conducted with spread 2% of inoculum onto 300 mL of Z^{-1}A agar medium and incubated at room temperature for 5 d. The Z^{-1}A agar medium was cut about 2x2 cm and macerated twice with ethyl acetate for 2 x 24 h then filtered. The filtrate was centrifuged at 4.000 x g for 30 m. The supernatant was concentrated by *rotary vacuum evaporator* and used for antimicrobial assay.

Cultivation of *Moraxella* sp. RA15 in broth medium was conducted with inoculated 700 µL of inoculum onto varying broth medium and incubated on a shaker medium at 110 rpm at 30 ^oC for 5 d. After incubated 2 d, the culture was added 4% of Amberlite XAD-16 and and incubated on a shake-incubator rotating 140 rpm at room temperature for five days. Furthermore, Amberlite XAD-16 was collected and put into the column and eluted with methanol.

Determination of Growth Curve

Growth curve of *Moraxella* sp. RA15 was conducted with transfer to the amount of inoculum onto ZoBell broth medium until OD600 of medium = 0.01. The culture was incubated on a shaker medium at 130 rpm at 30 ^oC. Read the OD_{600} of the cell suspension against Zobell broth blanks every 2 hours. In addition to Zobell broth, determination of the growth curve was determined for other medium namely 1/10 Z^{-1}, M13, and B1.

Antimicrobial Activity Assay for Bacteria Extract

Antimicrobial activity of bacteria extract did determined based on modified well-difusion agar methods (Valgas *et al.*, 2007). Well with diameter of 6 mm was made in plate containing nutrient agar media using a punch then its media was spreaded with inoculum of bacterial test. Furthermore the well was filled with 1,000 µg/well of the bacterium extract and incubated at 37 ^oC for 24 h. The extract having antimicrobial activity was signed with formation of inhibition zone around isolate then diameter of inhibition zone (measured from the edge of the colony to the edge of the clear zone) was recorded.

Parasite Strains and In Vitro Culture

P. falciparum FCR-3 (chloroquine-resistant with an IC_{50} of 130ng/mL) was cultured continously according to Trager and Jensen, 1976) with small modifications. The parasites were maintened *in vitro* in human erythrocytes (O±), diluted to 1% hematocrit in RPMI 1640 (Sigma) supplemented with 25 mM HEPES and 30 mM $NaHCO_3$ and complemented with 5% human O± serum. Parasites culture was incubated at 37°C in candle jars with a daily change of medium. Parasites cultures were synchronized with 5% of D-sorbitol given every 48 hours.

Antiplasmodial Assay

One hundred µL of culture parasite in condition at ring stages at 0.5-2% parasitemia (hematocrit 1%) were put in 96-well culture plates then added 50 µg/mL of *Moraxella* sp. extract. After incubation 48 h, the culture was prepared thin blood smear with giemsa stain and examined microscopically under oil immersion at 1,000 magnification to calculate 2,000 erythrocytes. Control was parasite without extract tested and its parasitemia was referred to as 100%. Inhibitory rate was calculated as follow:
Growth inhibiton of parasite = [(A-B)/A] x 100%
Where: A= percentage of parasitemia in control
B = percentage of parasitemia in sample

Brine Shrimp Lethality Test

Bacteria extract was screened for toxicity with larvae (nauplii) of *Artemia salina* (Piccardi *et al.,* 2000). The eggs were placed in an aerated bottle containing 33 g L-1 NaCl saline and natural lighting. After two days of hatching period at ambient temperature, 10 larvae of *A. salina* was mixed with bacterium extract with certain concentration namely 10, 100, 200, 400, 600, 800 and 1,000 µg/m L. The mixture was diluted with distilled water until 1 mL then incubated at ambient temperature for 24 hours. The amount total of survived larvae was counted under microscope and recorded. The data were analyzed by SPSS version 17 for probit analysis to determine LC50 values.

Herbicidal screening

Herbicidal screening was determined to detect extract bacteria inhibit De Novo starch synthesis according to modified Kida *et al.,* 1985). *Panicum repens* was growed in a green house a in ambient temperature. After planting (second leaf stage), the pot was moved to a dark room and kept for 12 h in ambient temperature. Part of the second leaf was cut into segments of about 5 mm. One of leaf segments were stained with 1 drop of iodine solution (0.2% I_2 in 2% KI). If result of stained leaf segment did not show a dark blue, the other segments can be transfered into a petridish containing 1.8 ml of the reaction medium and 0.2ml of a sample to be tested. The reaction medium consisted of 0.01 m potassium phosphate buffer (pH 6.5) and 0.01% (v/v) Tween-20 (a wetting agent). The Petridishes were illuminated at an intensity of 14 klux for 16hr at 25°C. The leaf segments were then removed, immersed in hot methanol for pigment extraction, and stained with iodine (a solution of 0.2% iodine and 2% potassium iodide). White or light yellow of leaf segment showed positive filtrate of herbicide.

RESULTS AND DISCUSSION

Identification of *R15 Isolate*

Identification results of isolate RA15 involved macroscopic, microscopic and biochemical characters were presented in Table 1. If it was compared with Manual for the Identification of Medical Bacteria or Bergey's Manual of Determinative Bacteriology, so isolate RA15 is genus Moraxella. Moraxella also was isolated from Acropora cerivernis in Kepulauan Bidong, Malaysia (*Kalimutho et al., 2007*).

Effect of solid media composition on production of antimicrobial compounds

In the preliminary screening, *Moraxella* sp. RA15 which was cultivated in different solid medium namely $Z^{-1}A$, $Z^{-1}GA$ and $Z^{-1}GLA$ for preliminary screening of antimicrobial activity. It was against directly with microorganism tests. As a result, it showing antimicrobial activity was only in $Z^{-1}GA$ medium (Table 1). Antimicrobial substances could be recovered from Z^{-1} GA medium, it can be inferred that the antimicrobial substance may be bound to the outer cell surface and secreted into the solid medium. This supported with earlier observations that the inhibitory compound remain closely bound to the cell (Rosenfeld and ZoBell, 1947; Lemos *et al.,* 1985; Abraham, 2004).

Difference of medium composition among $Z^{-1}A$, $Z^{-1}GA$ and $Z^{-1}GLA$ was only carbon sources. Carbon source contained $Z^{-1}GLA$ was glucose. Glucose was probably play a role in produce antimicrobial compounds from *Moraxella* sp. RA15. Glucose probably induced master genes coding production of antimicrobial substances from *Moraxella* sp. RA15. This phenomena was also exhibited in antimicrobial activity assay of *Flavobacterium* sp. K08, *Alteromonas* sp. K09, *Bacillus* sp. K10, *Klebsiella* sp. K11, and *Pseudomonas* sp. K12 (Nofiani *et al.,* 2009). However, studies have reported that glucose frequently inhibited the biosynthesis of many antibiotics (Huck *et al.,* 1991; Lounes *et al.,* 1995). *S. viridochromogenes ASA126* begin to synthesize avilamycin when at a low concentration of glucose (Zhu *et al.,* 2007). *Bacillus licheniformis* EI-34-6 required glycerol and $FeCl_3$ for synthesis of antimicrobial compounds (Yan *et al.,* 2003).

Effects of various media and growth condition on antimicrobial, antiplasmodial, cytotoxic and herbicidal activities

After the preliminary screening of antimicrobial activity, *Moraxella* sp. RA15 extract was prepared with cultivated in 2 growth conditions namely broth and solid medium and various media to evaluate various biological activities namely antimicrobial, antiplasmodial, toxic and herbicidal. The antimicrobial activity was only exhibited by the extract prepared in Z^{-1} GLA medium (Table 3). All of extracts produced various medium namely B1 broth, M13 broth, Z broth andZ^{-1}broth could

inhibit growth of *P. falcifarum* FCR3 but the best antiplasmodial activity was obtained by the extract produced in in Z^{-1} broth media (Table 3). Toxic activity was performed by BSLT assay using 200 ug/mL extracts produced B1 broth, M13 broth, Z^{-1}GA, and Z^{-1}. The best activities showed only with extract produced in Z^{-1} A medium with LC_{50} values namely 281.67 µg/mL which it was classified toxic. Based on BSLT result, antimicrobial activity of *Moraxella* sp. RA15 extract in Z^{-1} A medium is not possibly as an antitumor- *repens* due to inhibit *de novo* starch synthesis which it potents as a antimicrobial substances. *Moraxella* sp. RA15 extract in Z^{-1} A medium also showed herbicidal activity on the grass of *Panicum* candidate herbicide. Growth curve can be used to predict the best time of bacteria to produce secondary metabolites. Generally, secondary metabolites can be produced on stationer phase. Growth curve of *Moraxella* sp. RA15 exhibited that minimal medium can accelerate stationer phase. For example, Z^{-1} medium is the fastest to reach stationer phase than that of the other (Figure 1).

Secondary metabolites produced *Moraxella* sp. RA15 exhibited various biological activity in various medium and condition of bacterial production. It can be concluded that medium and condition are important factors in bioactive compound production from bacteria.

Table. 1: Characterization of Isolate RA15.

Evaluation	Result
Staining	
Gram test	negative
Cell shape	Short rod
Morphological Observation	
Motility	
Colony	Nonmotile
Shape	Positive
Elevation	
Edge	Circular
Color	Convex
Diameter	Flate
Biochemical Test	Putih kekuningan
Oksidase	4-5 mm
Catalase	Positive
Glucose Fermentation	Fermentative
Carbohydrate Catabolism	
a. Glucose	Negative
b. Lactose	Negative
c. Maltosa	Negative
d. Mannitol	Negative
e. Saccharose	Negatif
Decarboxylase	
a. Lysin	Negative
b. Arginine	Negative
c. Ornithine	Negative
Indole	Negative
Urease	Positive
H_2S	Positive
Citrate utilization	Negative
Growth test in TCBS medium	Negative

Table. 2: Screening of Antimicrobial Activity of *Moraxella* sp. RA15 on Different Media

Media	Microoganism test										
	1	2	3	4	5	6	7	8	9	10	11
Z^{-1}A	-	-	-	-	-	-	-	-	-	-	-
Z^{-1}GA	+	+	+	-	-	-	-	-	-	-	-
Z^{-1}GLA	-	-	-	-	-	-	-	-	-	-	-

Note : 1. *B. subtilis*; 2. *P. aeruginosa*; 3. *S.aureus*; 4. *E. coli*; 5. *V. vara*; 6. *V. cholerae*; 7. *V. harveyi*; 8. *Salmonella* sp.; 9. *A. hydrophila*; 10. *C. tropicalis*; 11. *C. albicans*. += positive test; - = negative test.

Table. 3: Activity Antimicrobial of the *Moraxella* sp. RA15 Extract on Different Media

Media	Microoganism test										
	1	2	3	4	5	6	7	8	9	10	11
Z broth	-	-	-	-	-	-	-	-	-	-	-
Z^{-1}GA	14,90±1,83	14,8±0,43	12,18±0,29	-	-	-	-	-	-	-	-
Z^{-1}broth	-	-	-	-	-	-	-	-	-	-	-
M13 Broth	-	-	-	-	-	-	-	-	-	-	-
B1 Broth	-	-	-	-	-	-	-	-	-	-	-

Note : 1. *B. subtilis*; 2. *P. aeruginosa*; 3. *S.aureus*; 4. *E. coli*; 5. *V. vara*; 6. *V. cholerae*; 7. *V. harveyi*; 8. *Salmonella* sp.; 9. *A. hydrophila*; 10. *C. tropicalis*; 11. *C. albicans*. - = negative test.

Table . 4: Antiplasmodial Activity of *Moraxella* sp. RA15 extract on Different Media

Media	Dosage (µg/mL)	Parasitemia (%)	Mortality (%)
B1 broth	100	10.12 ± 0.1928	32.44 ± 0.1082
M13 broth	100	4.50 ± 0.1844	69.90 ± 0.1730
Z broth	50	5.65 ± 0.2284	81.22 ± 0.3028
Z^{-1}broth	100	11.85 ± 0.11129	20.74 ± 0.1502

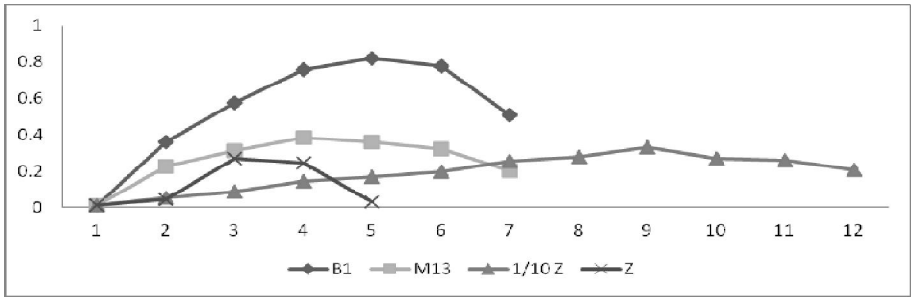

Fig. 1: Growth Curve of *Moraxella* sp. RA15 in Various Medium

REFERENCES

Abraham T.J. Antibacterial marine bacterium deterluminous vibriosis in shrimp larvae, Naga. World Fish Center Q. 2004;27: 28-31.

Bourne D.G., Munn C.B. Diversity of Bacteria Associated with the Coral *Pocillopora damicornis* from the Great Barrier Reef, Environ Microbiol. 2005;7:1, 1162-1174.

Gudbjarnason S. Bioactive marine natural product. Rit. Fiskideilar. 1999;16: 107-110.

Huck T.A., Porter N., Bushell M.E. Positive selection of Antibiotic-producing Soil Isolates. J Gen Microbiol. 1991;137:2321-2329.

Kalimutho M., Ahmad A., Kassim Z. Isolation, Characterization and Identification of Bacteria associated with Mucus of *Acropora cervicornis* Coral from Bidong Island, Terengganu, Malaysia, *Malaysian J. Sci.* 2007; 26: 27–39.

Kida T., Takano, S., Ishikawa T. A simple bioassay for herbicidal substance of microbial origin by determining de novo starch synthesis in leaf segments. Agric. Biol. Chem., 1985; 49: 1299-1303.

Koren O., Rosenberg E. Bacteria associated with mucus and tissue of the coral *Oculina patagnica* in summer and winter. Applied Environ. Microbiol., 2006;72: 5254-5259.

Lemos M.L., Toranzo A.E., Barja J.L. Antibiotic Activity of Epiphytic Bacteria Isolated from Intertidal Seaweeds. Microbiol. Ecol. 1985;11:149-163.

Lounes A., Lebrihi A., Benshlimane C. Regulation of valine catabolism by ammonium in *Streptomyces ambofaciens*: Effect of glucose and inorganic phosphate. Applied Microbiol. Biotechnol. 1995;45: 204-211.

Nofiani R., Kadarisno., Daryati., Sapar A. *Characteristics of antimicrobial activity of Eucheuma cottonii doty-associated bacteria extracts*. J. Pengolahan Hasil Perikanan Indonesia 2009;12: 148-158.

Okami Y. Potential use of marine microorganisms for antibiotics and enzyme production. Pure Appl. Chem. 1982; 54: 1951-1962.

Piccardi R., Frosini A., Tredic M., Margheri M. Bioactivity in free-living and symbiotic cyanobacteria of the genus Nostoc, *J. Appl. Phycology*. 2000;12: 543-547.

Ritchie K.B. Regulation Of Microbial Populations by Coral Surface Mucus and Mucus-Associated Bacteria, *Mar. Ecol. Prog. Ser.* 2006; 322: 1–14.

Radjasa O.K., Salasia S.I.O., Sabdono A., Weise J., Imhoff J.F., Lammler C., Risk M.J. Antibacterial Activity of Marine Bacterium *Pseudomonas* sp. Associated with Soft Coral *Sinularia polydactyla* against *Streptococcus equi* Subsp. *zooepidermicus*, Intl. J. Pharmacol. 2007; 3(2):170-174.

Rohwer F., Seguritan V., Azam F., Knowlton N. Diversity and Distribution of Coral-associated Bacteria, *Mar. Ecol. Prog. Ser.* 2002;243: 1-10.

Rosenfeld W.D., Zobell C.E. Antibiotic production by marine microogansims. J. Bacteriol. 1947;54: 393-398.

Shnit-Orland M., Kushmaro A. Coral mucus bacteria as a source for antibacteria activity. Proceedings of the 11th International Coral Reef Symposium. Ft. Lauderdale, Florida, 2008; 257-259.

Trager W., Jensen J.B. Human malaria parasites in countinous culture. Sci. 1976;193: 673-675.

Yan L., Boyd K.G., Adams K.G., Burgess J.G., Biofilm-specific cross-species induction of antimicrobial compounds in bacilli. Applied Environ. Microbiol. 2003;69: 3719-3727.

Zhu C., Lu F., He Y., Han Z., Du L., Regulation of avilamycin biosynthesis in *Streptomyces viridochromogenes*: Effect of glucose, ammonium ion and inorganic phosphate. Appl. Microbiol. Biotechnol. 2007;73: 1031-1038.

Acknowledgments

We would like thank you for Higher Education Directorate which was funded the research through Young Lecturer Research Grant by DIPA grant number 308/SP2H/PP/DP2M/III/2008 on March 6th, 2008 and Collaboration Research Grant in 2008.

How to cite this article:

Risa Nofiani, Rizmahardian Ashari Kurniawan, Tika Mabella, Puji Ardiningsih, Titin Anita Zahara and Sunarto**.** Study of Media Effect toward Antimicrobial, Antiplasmodial, Herbicidal Activities and Toxicity of Extract Produced by *Acroporadibranchiata-Associated Moraxellasp. RA15*. J App Pharm Sci. 2012; 2(9): 010-014.