ISSN 1996-0700

Asian Journal of Biotechnology

Knowledgia
SCIENTIFIC
A Place to Publish Outstanding Research

http://knowledgiascientific.com

Asian Journal of Biotechnology 5 (1): 21-32, 2013
ISSN 1996-0700 / DOI: 10.3923/ajbkr.2013.21.32
© 2013 Knowledgia Review, Malaysia

Antibacterial Properties of *Rhodobacteracea bacterium* Sp.2.11 Isolated from Sponge *Aaptos aaptos* Collected from Barrang Lompo East Sulawesi

[1,3]Tutik Murniasih, [1]Soleh Kosela, [2]L.B.S. Kardono and [1]Wahyudi Priyono
[1]Department of Chemistry, Faculty of Mathematics and Natural Science, University of Indonesia, Indonesia
[2]Research Center for Chemistry, Indonesian Institute of Sciences, Indonesia
[3]Research Center for Oceanography, Indonesian Institute of Sciences, Indonesia

Corresponding Author: Tutik Murniasih, Department of Chemistry, Faculty of Mathematics and Natural Science, University of Indonesia, Indonesia

ABSTRACT

Marine sponge *Aaptos-aaptos* is known as antimicrobial compound aaptamine and its derivatives producer. To establish whether associated bacteria were the truly produced bioactive compounds, for preliminary, study of antimicrobial properties was done to know the potency of antimicrobial production in various carbon source. In this research, approximately 10 bacterial colonies were isolated from sponge *Aaptos aaptos*. Among isolated bacteria, colony Sp.2.11 was the most potential producing antibacterial compound. The partial gen 16S-rDNA sequencing of this bacteria showed 99% similarity with *Rhodobacteracea bacterium* species. Extra and intra cellular bacterial extract of this strain strongly inhibited the growth of pathogenic bacteria *Staphylococcus aureus* and *Vibrio eltor* while moderately against *Bacillus subtilis*. Optimization of activity was conducted by culturing *Rhodobacteraceae bacterium* in various carbon sources. Anti-gram positive bacterial production of extra cellular bacterial extract was highest when culturing this bacteria using minimum carbon source containing pepton, yeast extract in sea water medium (SYP), while anti gram negative bacterial was maximum in SYP medium with glucose as carbon source. Anti-gram positive bacterial production of intra cellular bacterial extract was highest in SYP with glycerol as carbon source, while anti-gram negative was highest in SYP with glucose as carbon source. In this research the highest biomass production didn't increase the antibiotic production.

Key words: Antibacterial, *Rhodobacteracea bacterium, Aaptos aaptos*, symbiotic bacteria

INTRODUCTION

Sponges are considered as one of the most important marine source of pharmacologically active substances. Since, developing of bioactive substances derived from sponge faced many problems on mass production, the study on bioactive compounds derived from associate bacteria is growing rapidly. Piel *et al.* (2004) and Thiel and Imhoff (2003) cite in Wang (2006) reported that some bioactive substances derived from sponges were produced by associate microorganisms. Among the origin of associate microorganisms, the highest number (33%) of bioactive compounds, were derived from sponge-associate microorganisms (Kelecom, 2002). Microorganisms serve sponge's food, provide a mutual symbiotic inter and intra cellular (Friedrich *et al.*, 1999; Vacelet and Donadey, 1977; Muscholl-Silberhorn *et al.*, 2008). Symbiotic bacteria also play an important role in epibacterial

defense of the host (Holmstrom *et al.*,1996; Boyd *et al.*, 1999) cite in (Thakur *et al.*, 2004, Holmstrom and Kjelleberg, 1999; Thakur and Anil, 2000). Study on *Suberitas domuncula* suggested that sponge in providing chemical defense could be produced antibacterial metabolite directly or indirectly produce by epibacteria defense. In fact, Bewley *et al.* (1996) and Unson *et al.* (1994) reported these secondary metabolites migh have a function as antibacterial (Schmidt *et al.* 2000), antifungal (Bewley *et al.*, 1996), cytotoxic (Muscholl-Silberhorn *et al.*, 2008). Compounds produced by marine microorganisms are promising as long can be produced both for analysis and for large-scale production. Some of the efforts are by optimizing the fermentation process (Hirose, 2003).

Research on the potential of *Aaptos*-associate bacteria was done by Radjasa *et al.* (2007). It was reported that the screening of *Aaptos*-associate bacteria for antibacterial substances and MDR (Multi Drug Resistance) showed three potential bacteria, namely, *Aquamarina halomonas* species, *α proteobacterium* and *Pseudoalteromonas luteviolacea*. In addition Kencana and Hutagalung (2008) also reported that three groups of bacteria isolated from the sponge *Aaptos* from northern Java Sea showed anti-MDR activity. This study reported research on *Aaptos*-associate *Rhodobacteraceae bacterium* for antibacterial agent.

MATERIALS AND METHODS

Collection of bacteria: *Aaptos* sp. sponge was collected by scuba diving at 2-10 m depth from the Barranglompo Island, South Sulawesi in June 2009. Isolation of bacteria were carried in fresh state (direct plating) at the sampling location. Study of selected bacteria *Rhodobacteracea bacterium* for antibiotic was done since 2010. The method used for isolation of sponge-associated bacteria was done using direct plating method (Hirose, 2003; Devi *et al.*, 2010). Method of "direct plating", was performed in the following manner: A piece of sponge is inserted in the 5% marine broth media in sterile sea water and stirred, then performed dilution. Concentration of each dilution was taken 100 μL dropwise in 10% of marine agar media in order to be deployed and incubated at 30°C.

Isolation and purification of potential bacteria: The observation was conducted for the growing colonies after incubated for one to two weeks. The number of colonies were counted and a different morphology (shape, color) of colonies, isolated and moved to the agar media. Each colony was purified to obtain a single bacterial colony. All of isolated bacteria stored using gliserol stock in freezer for further investigation.

Screening of antibiotic-producing bacteria: All isolates bacteria were cultured in 5 mL of marine broth medium (100%), then incubated for 5 days using shaker (100 rpm). Cultures broths were centrifuged in 6000 rpm at 4°C for 15 min and separated between pellet and supernatant. Extraction was performed both for the supernatant and pellet. Supernatant was extracted using 5 mL of ethyl acetate, then evaporated to remove the organic phase, dissolved and extracted using methanol. The pellets were extracted using acetone, centrifuged in 6000 rpm at 4°C for 15 min, removed the acetone and dissolved in methanol.

Screening of antibacterial activities was conducted to the supernatant and pellet extract of isolated bacteria. The pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Vibrio eltor* were use for bioindicator. The three of pathogenic bacteria were obtained from the Department of Microbiology, Faculty of Medicine, University of Indonesia. Antibacterial screening method used in this research was agar diffusion method (Bauer *et al.*, 1966). Approximately 15 μL sample was

dropped on the 6 mm paper disk, evaporated in a “clean bench” until dry then deposited on the surface of nutrient agar medium that previously inoculated with pathogenic bacteria. The positive control used in this research was 10 µg ampicillin. Incubation was carried out at 30°C for one night. The antibacterial activity was shown by the clear zone around the paper disk and compared with the activity of the antibiotic ampicillin.

Identification of selected bacteria: Identification of bacteria was done using molecular method, by analyzing the partial 16SrDNA gene. 16SrDNA gene was amplified using primers 9F (5'-AGRGTTTGATCMTGGCTCAG-3') and 1492R (1492R: 5'-TACGGYTACCTTGTTAYGACTT-3'). The reaction conditions are, 95°C, 2 min (1 cycle), 95°C, 30 sec, 65°C, 1 min, 72°C, 2 min (10 cycle), 95°C, 30 sec, 55°C, 1 min, 72°C, 2 min (30 cycle) and 72°C, 2 min (1 cycle).

Sequencing of 16SrDNA gene were analyzed using DNA sequencer from the automatic machine Bioscience Charoen Pok Phan laboratory. The amino acid base pattern then compare to the GeneBank/DDBJ/EMBL based on BLAST (Altschul *et al*., 1997).

Nutrition optimization of selected bacteria: Optimization of antibiotic production was done by fermenting the bacteria in various carbon source (Hirose, 2003). Variation 1 consisted of 100 mL of SYP (0.1 g yeast extract, 0.5 g peptone) in sea water and 1 mL of selected bacterial without any addition of a carbon source. Variation 2 media consisted 100 mL SYP, 1 mL of bacterial culture and 1 g of glucose. Variation 3 consisted of 100 mL medium SYP, 1 mL of bacterial culture and 1 g maltose. Variation 4 consisted of 100 mL of SYP medium, 1 mL bacterial culture, 1 g of lactose while the variation 5 consisted of 100 mL SYP media, 1 mL bacterial culture and 1 g glycerol. The solution was incubated at room temperature using the "shaker" for 4 days. Each experiment performed 3 times (triplo). Culture solution was sampled approximately 5 mL at day 0, 1, 2, 3 and 4. UV-vis absorbance was measured at a wavelength of 660 nm for determining the growth and extracted for antibacterial testing.

Determination of biomass concentration: A fix volume of culture collection that harvesting at day-0,1,2,3 and 4 were centrifuged at 6000 rpm, 4°C during 10 min. The pellet and supernatant were separated. Measure the weight of pellet after centrifugation and after drying. Drying of pellet was done at 110°C during 24 h. DCW (Dried Cell Weight) was determined as gram dried pellet per liter culture collection.

Determination of antibacterial activity: Antibacterial activity of supernatant and pellet extract of *Rhodobacteraceae bacterium* against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative bacteria (*Vibrio eltor*) were conducting using diffusion method (Bauer *et al*., 1966). Maxwell *et al*. (1994) noted that antibiotic activity was expressed as unit of activity per milliliter of supernatant. One Unit (U) as defined as 1 mm of annular clearing zone around antibiotic disk (Wang *et al*., 2011).

RESULTS

Symbiotic bacteria isolation: Isolation was done using “direct plating” by stocking the samples directly at the field. In the sponge *Aaptos* sp., the values of CFU mL^{-1} was 2.4×10^5, bacteria then subsequently was stored in glycerol stock to avoid the change.

Table 1: Results of antimicrobial screening of *Aaptos* sp-associated bacteria

Host	Bacteria code	Inhibition zone against (mm)		
		S. aureus	*B. subtilis*	*V. eltor*
Sponge Sp2 (*Aaptos* sp.)	Sp2.1	8 (s)	-	-
	Sp2.2	8 (s)	-	-
	Sp2.3	8 (s)	-	-
	Sp2.5	8 (p)	-	8 (s)
	Sp2.6	9 (p)	-	-
	Sp2.7	-	8 (s)	10 (s)
	Sp2.8	14 (p),12 (s)	8 (s)	8 (p), 9 (s)
	Sp2.9	15 (p)	-	7 (p)
	Sp2.10	-	-	7 (p), 10 (s)
	Sp2.11	15 (p), 20 (s)	11 (s)	13 (s)

s: Supernatant, p: Pellet

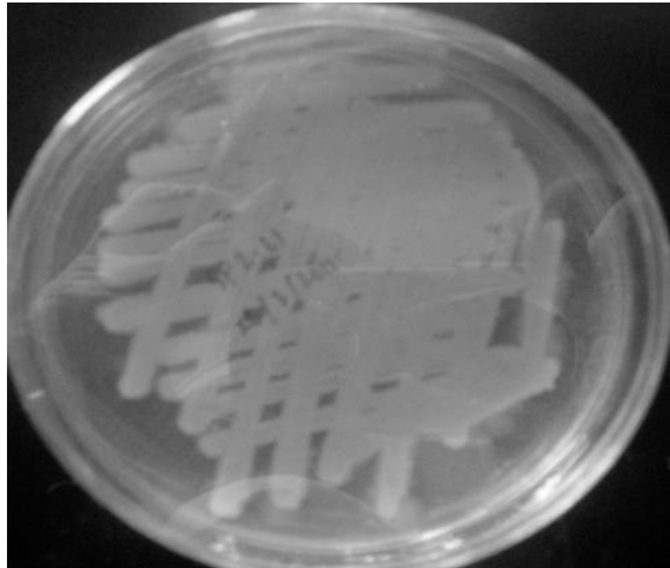

Fig. 1: Profile of potential bacteria Sp.2.11

Antibacterial activity screening: Screening of antibacterial activity toward several sponge-associate bacteria extracts against pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Vibrio eltor* indicated that there were some extracts inhibited the growth of pathogenic bacteria.

Table 1 showed that the highest antibacterial activity was reach by Sp.2.11 bacteria with the zone inhibition of pellet extract against *Staphyococcus aureus* was 15 mm, while, in turn, the supernatant extract of *Staphyococcus aureus* was 20 mm, 11 mm against *Bacillus subtilis* and 13 mm against *Vibrio eltor*.

Bacteria identification: Potential bacteria Sp2.11 isolated from sponge (*Aaptos* sp.) was cream in color, wide-legged colonies. Figure 1 is the profile of the isolated Sp2.11 bacteria.

Following was the result of base patern of 16SrDNA sequencing:

colony_16S_F:
CNATGGCAGACGGGNTGAGTAACGCGGTGGGAAGCTACCTTGTGGTAGGGAACAACAGTT
GAAACGACTGCTAATACCCTATGAGCCCTATGGGGGAAAGATTTATCGCCATGAGATGTGG
TCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGAGTGATGACG
GCCTTAGGGTTGTAAAGCTCTTTCAGCAGTGAAGATAATGACATTAACTGCAGAAGAAGCCCC
GGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATCACT
GGGCGTAAAGCGTACGTAGGCGGACTGATCAGTCAGGGGTGAAATCCCGGGGCTCAACCC
CGGAACTGCCTTTGATACTGTCAGTCTTGAGATCGAGAGAGGTGAGTGGAACTCCGAGTGT
AGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGCTCGA
TACTGACGCTGAGGTACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGAATGCTAGTTGTCAGGTANCTTGCTATTTGGTGACNCACCTACGCATTA
NCATTCCCCCTGGGGAGTACGGTCNCAANATNAAACTCAAAGGAATTGACGGGGGCCCCCC
CAANCGGTGGANCATGTGGTTTAATNCNAACAACNCNCAAAACNTACCACCCTTGANTTTG
GCCCTCCAACNGAAACGGTGGNTNCCNTTNGGGANNNCNGNNANGNNCNNCNGGCTNNC
NCCCCCCNNNCNGNAANNTNGGTTNANNCCCCNCNANCCANCCCCCCCNTNTTNCCNCCT
TTNTTNGNCCCNNNGGGNNCNCCNNNNAAANCCNA
GCCCGCGTTAGATTAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCGACGATCTATAGCT

colony_16S_R:
ATGNGTTGGCATGCATGTTNGCGATTACTAGCGATTCCAACTTCATGCTCTCGAGTTGCA
GCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACA
CGGTTGTAGCGCCAAATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACC
ACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCG
TACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGTCACCAAATAGCAAGCTACCTGAC
AACTAGCATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCA
CGCTTTCGTACCTCAGCGTCAGTATCGAGCCAGTGAGCCGCCTTCGCCACTGGTGTTCTT
CCGAATATCTACGAATTTCACCTCTACACTCGGAGTTCCACTCACCTCTCTCGATCTCAA
GACTGACAGTATCAAAGGCAGTTCCNGGGGTTGANCCCCCGGGAATTNCACCCCTGANTG
ATCAGTCCGCCTACGTACGCTTTACCCCAGTGATCCGAAAACGCTAGCCCCTTCGTATNA
CGCGGCTGCTGGCACNAANTTANCNGGGCTTCTTCNGCATTAANNCATNATNTNCCTGNT
GAAAAACTTTNANCCTAAGGCGTCNTCCTCACCGGCAGGCTGNNAAGGNTGCCCCATNTC
AANTCCCATGNTNCCCCCNAAAANTNGGCCNGTTN

Sequencing of 16SrDNA analysis results was shown at the previous paragraph. Analysis of similarity with the Gene Bank data base using the Blast Sp2.11 bacteria, showed this bacteria having 99% similarity to the *Rhodobacteraceae bacterium* 1tc14 16S ribosomal RNA gene. Rhodobacter is classed to α Proteobacter bacteria. Radjasa *et al*. (2007) isolated *Aaptos*-associated bacteria that have 100% similar to α Proteobacterium D21.

Optimization of antibacterial production: Figure 2 was the growth of *Rhodobacteraceae bacterium* in several carbon source medium.

The growth curve's patern in each type of carbon source was quite similar which is the log phase obtained at 1-2 days incubation, stationary phase was showed after 2 days incubation. The highest biomass that described by optical density is reach when culturing

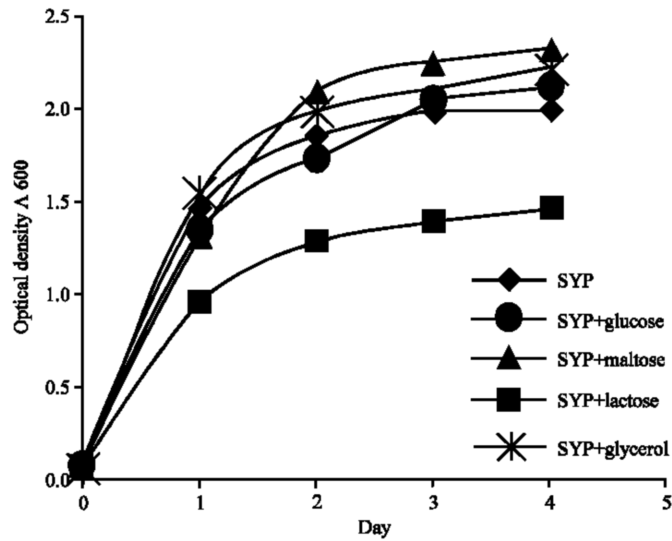

Fig. 2: Growth of *Rhodobacteraceae bacterium* Sp2.11 in various carbon source

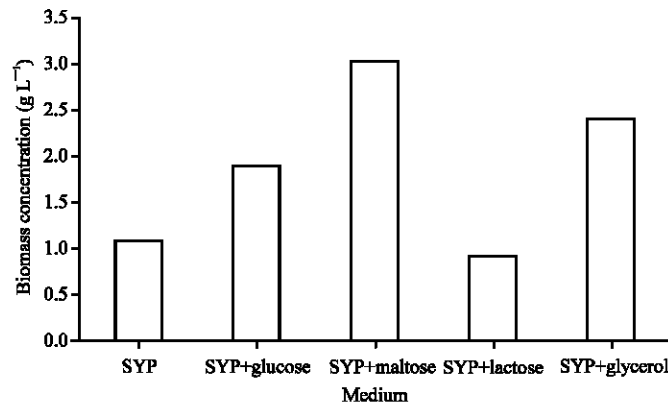

Fig. 3: Biomass concentration at fourth day cultivation

Rhodobacteracea bacterium using SYP and maltose as carbon source media. Biomass concentration at fourth day harvesting antibacterial of supernatant extracts in various carbon sources in Fig. 3-9.

Biomass concentration at 4th day harvesting was highest when cultivate Sp2.11 in SYP medium with maltose carbon source. This data will be compare to the antibacterial activity as shown in Fig. 4-9.

Figure 4-6 described the supernatant extract of *Rhodobacteracea bacterium* Sp. 2.11 againts gram positive and negative phatogenic bacteria. Activity of supernatant against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* were maximum by cultivating *Rhodobacteraceae bacterium* Sp.2.11 in SYP medium without any addition of carbon sources (maltose, glucose, lactose and glycerol). Culturing this bacteria in SYP medium produced substances which have 5 U Anti *Staphylococcus aureus* activity (Fig. 4) and 6,2 U Anti *Bacillus subtilis* activity (Fig. 5) per mililiter bacterial supernatant. Activity of supernatant against gram negative bacteria *Vibrio eltor* maximum in the cultivation using SYP with glucose as carbon source (7,8 U mL^{-1}), otherwise cultivating in the minimum medium SYP without carbon source also showed high activity (7,6 U mL^{-1}) (Fig. 6).

The effect of carbon source to the antibacterial production of pellet (intra cellular) described in Fig. 7-9. Figure 7 showed the antibacterial activity of pellet extract against *Staphylococcus aureus*,

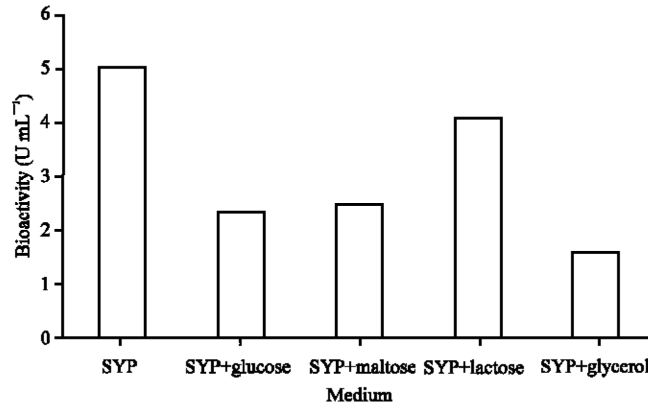

Fig. 4: Effect of carbon source to the supernatant activity against *Staphycoccus aureus*

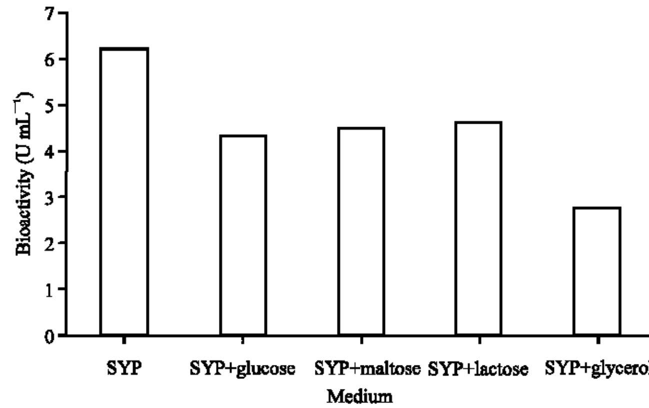

Fig. 5: Effect of carbon source to the supernatant activity against *Bacillus subtilis*

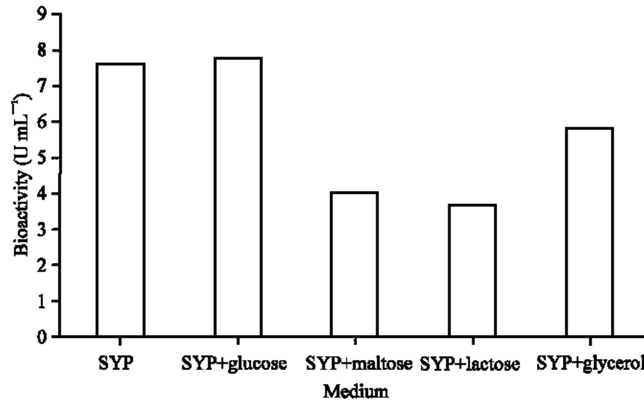

Fig. 6: Effect of carbon source to the supernatant activity against *Vibrio eltor*

Bacillus subtilis (Fig. 8) and *Vibrio eltor* (Fig. 9). Activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* are highest when cultivating *Rhodobacteraceae bacterium* in SYP medium with glycerol addition. Activity of pellet in SYP+glycerol medium against *Staphylococcus aureus* is approximately 4,6 U mL^{-1} and

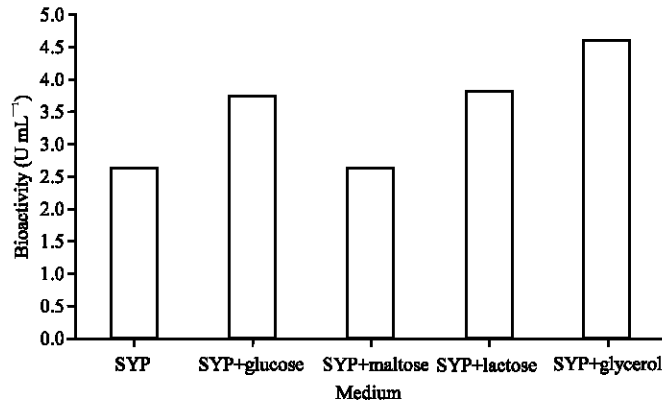

Fig. 7: Effect of carbon source to the pellet activity against *Staphylococcus aureus*

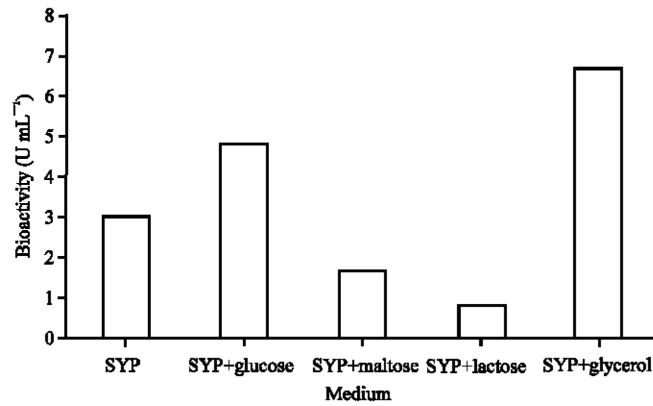

Fig. 8: Effect of carbon source to the pellet activity against *Bacillus subtilis*

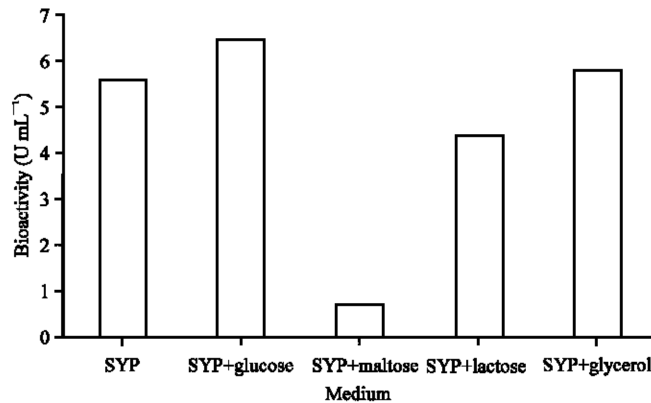

Fig. 9: Effect of carbon source to the pellet activity against *Vibrio eltor*

Bacillus subtilis is 6,67 U mL^{-1}. Similar study reported by Wang *et al.* (2011) that work with *Xenorhabdus bovienii*. Adding glycerol as carbon source to the medium culture of *Xenorhabdus* b. could improve the antibiotic activity. Activity of pellet against gram negative bacteria *Vibrio eltor* was highest in SYP medium with glucose addition. Activity of pellet in this medium against *Vibrio eltor* was 5,73 U mL^{-1}.

DISCUSSION

Antibacterial activity optimizing by adding carbon source to SYP medium was intended to obtain the highest production of antibacterial substances. In this experiment we observed the growth and the antibacterial properties of *Rhodobacteraceae bacterium* Sp2.11 against pathogenic gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative bacteria (*Vibrio eltor*) in various carbon source. Demain (1974) noted carbon source play an important role to control of existing secondary metabolism (Kennel and Demain, 1979).

The highest biomass production was reached by culturing *Rhodobacteracea bacterium* in SYP+maltose medium that contained yeast extract and pepton as nitrogen source and maltose as carbon source. Comparing between growth and biomass concentration showed that the highest biomass production was reached by culturing *Rhodobacteraceae bacterium.* in SYP+maltose. This result also significant with the biomass concentration at fourth day harvesting that highest by culturing this bacteria in SYP+maltose, which is approximately produce biomass concentration 3.027 g L^{-1}. The production of antibacterial substances was observed on stationary phase refered to previous study which was prove that production of secondary metabolite occure at stationary phase (Gesheva *et al.*, 2005). In this research we observed the production of antibacterial metabolite at fourth day harvesting.

The production of anti-gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* by extra celullar metabolite of *Rhodobacteraceae bacterium* culturing in various carbon source showed the similar pattern. The highest production was reach by culturing in SYP medium without adding the carbon source. This phenomenon was similar to the previous studies reported by Demain *et al.* (1983), Doull and Vining (1990) and Sanchez and Demain (2002) which proved that nutrient deficiency such as carbon and nitrogen is responsible for onset of antibiotic biosynthesis (cite in Gesheva *et al.*, 2005). The activity of extracellular metabolite against gram negative bacterial (*Vibrio eltor*) showed different pattern to the gram positive activity. Activity of supernatant metabolite against *Vibrio eltor* highest (7.8 U mL^{-1}) when culturing *Rhodobacteraceae bacterium* in SYP medium with glucose as carbon source. In this case glucose encourage the production of substances that play an impotant role to inhibit gram negative bacteria.

The effect of various carbon source to the pellet antibacterial production (intra cellular) that described in Fig. 7-9 showed different pattern against gram negative and gram positive activity. There are different compound that play an important role inhibit gram negative and gram positive bacteria in pellet and supernatant extract.

Adding glycerol as carbon source will increasing activity of antigram positive in pellet extract. Some antibiotics production such as niphimycin was increased by adding glycerol in culture medium (Georgieva-Borisova, 1974; Smith and Chater, 1988; Gesheva *et al.*, 2005).

The same data showed by extra cellular and intra cellular metabolite activity against gram negative bacteria that highest when culturing *Rhodobacteraceae bacterium* in SYP medium with glucose as carbon source. It indicate that there is a relation compounds that produce by extra and intra celullar which is play an important role to inhibit gram negative bacteria. In this case glucose encourage the production of substances that play an impotant role to inhibit gram negative bacteria. Glucose was a central carbon that induced several antibiotic production such as penicillin by *Penicillin chrysogenum* (Christense and Nielson, 2000), cephalosporine (Thykaer *et al.*, 2002; Nielsen and Eliasson, 2005).

CONCLUSION

The *Aaptos* sp-associated bacteria Sp2.11 was the most potential producing antibacterial compound. The partial gen 16S-rDNA sequencing of this bacteria showed 99% similarity with *Rhodobacteracea bacterium* species. Extra and intra cellular bacterial extract of this strain strongly inhibited the growth of pathogenic bacteria *Staphylococcus aureus* and *Vibrio eltor* while moderately against *Bacillus subtilis*.

Optimization of the growth showed the highest production of biomass was reached by culturing *Rhodobacteraceae bacterium* in SYP medium with maltose as carbon sources at fourth day harvesting.

Anti-gram positive bacterial production of extra cellular bacterial extract was highest when culturing this bacteria using minimum carbon source containing pepton, yeast extract in sea water medium (SYP) only without adding carbon source. The anti gram negative bacterial was maximum in SYP medium with glucose as carbon source. Anti-gram positive bacterial production of intra cellular bacterial extract was highest in SYP with glycerol as carbon source, while anti-gram negative was highest in SYP with glucose as carbon source.

Comparing between growth with antibiotic production, in this study the highest biomass concentration (reach by SYP+maltose) didn't increase the antibiotic production.

The effect of carbon source to the antibiotic production by *Rhodobacteraceae bacterium* was first reported in this study. Further investigation of antibiotic compounds will be conducted for our further work.

ACKNOWLEDGEMENT

We would like to thank to Bioscience Laboratory of Charoen Pok Pahn that provide laboratory for bacteria identification.

REFERENCES

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res., 25: 3389-3402.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bewley, C.A., N.D. Holland and D.J. Faulkner, 1996. Two classes of metabolites from *Theonella swinhoei* are localized in distinct populations of bacterial symbionts. Experientia, 52: 716-722.

Boyd, K.G., D.R. Adams and J.G. Burgess, 1999. Antibacterial and repellent activities of marine bacteria associated with algal surfaces. Biofouling: J. Bioadhesion Biofilm, 14: 227-236.

Christense, B. and J. Nielson, 2000. Metabolic network analysis on *Penicillium chrysogenum* using ^{13}C-labelled glucose. Biotechnol. Bioeng., 68: 652-659.

Demain, A.L., 1974. Biochemistry of penicillin and cephalosporin fermentations. Lloydia, 37: 147-167.

Demain, A.L., Y. Aharonowitz and J.F. Martin, 1983. Metabolite Control of Secondary Biosynthetic Pathways. In: Biochemistry and Genetic Regulation of Commercially Important Antibiotic, Vining, L.C. (Ed.). Addison-Wesley, London, UK., pp: 49-67.

Devi, P., S. Wahidullah, C. Rodrigues and L.D. Souza, 2010. The sponge-associate bacterium *Bacillus licheinformis* SAB1: A source of antimicrobial compounds. Mar. Drugs, 8: 1203-1212.

Doull, J.L. and L.C. Vining, 1990. Nutritional control of actinorhodin production by *Streptomyces coelicolor* A3(2): Suppressive effects of nitrogen and phosphate. Appllied Microbiol. Biotechnol., 32: 449-454.

Friedrich, A.B., H. Merkert, T. Fendert, J. Hacker, P. Proksch and U. Hentschel, 1999. Microbial diversity in the marine sponge *Aplysinia cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence in situ hybridization (FISH). Mar. Biol., 134: 461-470.

Georgieva-Borisova, J.C., 1974. Taxonomic characteristics of strain *Actinomyces hygroscopicus* B-255 and conditions for its antibiotic production. Ph.D. Thesis, University of Sofia, Bulgaria.

Gesheva, V., V. Ivanova and R. Gesheva, 2005. Effect of nutrients on the production of AK-111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. Microbiol. Res., 160: 243-248.

Hirose, Y., 2003. Personal communication. Training Course of Conservation and Sustainable Use of Tropical Marine Biological Diversity at Marine Biotechnology Institute Co. Ltd., Kamaishi Laboratory Japan.

Holmstrom, C. and S. Kjelleberg, 1999. Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. FEMS Microbiol. Ecol., 30: 285-293.

Holmstrom, C., S. James, S. Egan and S. Kjelleberg, 1996. Inhibition of common fouling organisms by marine bacterial isolates with special reference to the role of pigmented bacteria. Biofouling: J. Bioadhesion Biofilm Res., 10: 251-259.

Kelecom, A., 2002. Secondary metabolites from marine microorganisms. An. Acad. Bras. Cienc., 74: 151-170.

Kencana, D.S. and R.A. Hutagalung, 2008. Growth Inhibition of Clinically resistant bacteria by marine bacteria associate with sponge *Aaptos* sp. J. Coastal Dev., 11: 81-88.

Kennel, Y.M. and A.L. Demain, 1979. Effect of carbon source on ß lactam antibiotic formation by *Cephalosporium acremonium*. Exp. Mycol., 2: 234-238.

Maxwell, P.W., G. Chen, J.M. Webster and G.B. Dunphy, 1994. Stability and activities of antibiotics produced during infection of the insect *Galleria mellonella* by two isolates of *Xenorhabdus nematophilus*. Applied Environ. Microbiol., 60: 715-721.

Muscholl-Silberhorn, A., V. Thiel and J.F. Imhoff, 2008. Abundance and bioactivity of cultured sponge-associated bacteria from the Mediterranean sea. Microb. Ecol., 55: 94-106.

Nielsen, J. and A. Eliasson, 2005. From Glucose to Antibiotics: What Control the Fluxes? In: Biocombinatorial Approaches for Drug Finding, Wohlleben, W., T. Spellig and B. Muller-Tiemann (Eds.). Springer, New York, ISBN-13: 9783540220923, pp: 195-214.

Piel, J., D. Hui, G. Wen, D. Butzke, M. Platzer, N. Fusetani and S. Matsunaga, 2004. Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. Proc. Nation. Acad. Sci. USA., 101: 16222-16227.

Radjasa, O.K., D.H. Kencana, A. Sabdono, R.A. Hutagalung and E.S. Lestari, 2007. Antibacterial acivity of marine bacteria associated with sponge *Aaptos* sp. against Multi Drug Resistant (MDR) strain. J. Matematika Sains, 12: 147-151.

Sanchez, S. and A.L. Demain, 2002. Metabolic regulation of fermentation processes. Enzyme Microb. Technol., 31: 895-906.

Schmidt, E.W., A.Y. Obraztsova, S.K. Davidson, D.J. Faulkner and M.G. Haygood, 2000. Identification of the antifungal peptide-containing symbiont of the marine sponge *Theonella swinhoei* as a novel Deltaproteobacterium, BCandidatus *Entotheonella palauensis*. Mar. Biol., 136: 969-977.

Smith, C.P. and K.F. Chater, 1988. Cloning and transcription analysis of the entire glycerol utilization (*gylABX*) operon of *Streptomyces coelicolor* A3 (2) and identification of a closely associated transcription unit. Mol. Gen. Genet., 211: 129-137.

Thakur, N.L. and A.C. Anil, 2000. Antibacterial activity of the sponge *Ircinia ramosa*: Importance of its surface-associated bacteria. J. Chem. Ecol., 26: 57-71.

Thakur, N.L., A.C. Anil and W.E.G. Muller, 2004. Culturable epibacteria of the marine sponge *Ircinia fusca*: Temporal variations and their possible role in the epibacterial defense of the host. Aquat. Microb. Ecol., 37: 295-304.

Thiel, V. and J.F. Imhoff, 2003. Phylogenetic identification of bacteria with antimicrobial activities isolated from Mediterranean sponges. Biomol. Eng., 20: 421-423.

Thykaer, J., B. Christensen and J. Nielsen, 2002. Metabolic network analysis of an adipoyl-7-ADCA-producing strain of *Penicillium chrysogenum*: Elucidation of adipate degradation. Metab. Eng., 4: 151-158.

Unson, M.D., N.D. Holland and D.J. Faulkner, 1994. A brominated secondary metabolite synthesized by the cyanobacterial symbiont of marine sponge and accumulation of the crystalline metabolites in the sponge tissue. Mar. Biol., 119: 1-11.

Vacelet, J. and C. Donadey, 1977. Electron microscope study of the association between some sponges and bacteria. J. Exp. Mar. Biol. Ecol., 30: 301-314.

Wang, G., 2006. Diversity and biotechnological potential of the sponge-associated microbial consortia. J. Ind. Microbiol. Biotehnol., 33: 545-551.

Wang, Y., X. Fang, F. An, G. Wang and X. Zhang, 2011. Improvement of antibiotic activity of *Xenorhabdus bovienii* by medium optimization using response surface methodology. Microb. Cell Factories, Vol. 10. 10.1186/1475-2859-10-98.