Original Article

Bulletin of Environment, Pharmacology and Life Sciences

Online ISSN 2277-1808
Bull. Env. Pharmacol. Life Scien. Volume 1 [11] October 2012: 01- 07

Website: www.bepls.com

Antimicrobial Activity of Some Bacterial Isolates from Sibolangit Natural Recreational Park of North Sumatra, Indonesia

Dwi Suryanto*, Siti Khadijah Nasution & Erman Munir
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jln. Bioteknologi No. 1, Medan, Indonesia 20155.
*Email: suryantodwi0904@yahoo.com

ABSTRACT

A study on assay of antimicrobial activity of some bacterial isolates of Sibolangit Natural Recreational Park of North Sumatra, Indonesia was conducted. Bacterial characterization was done by microscope observation and simple biochemical tests. To extract antimicrobial compounds from selected bacterial isolates, organic solvents such as methanol, ethyl acetate, and n-hexane were used. To test antimicrobial activity of the extract, disc diffusion method was conducted using Gram-negative Escherichia coli, Gram-positive Staphylococcus aureus, and fungi Candida albicans, *Fusarium oxysporum and Ganoderma boninense as test microorganisms.*

Twenty-nine isolates indicated to inhibit bacterial and fungal growth when isolated from soil samples. Bacterial ability to inhibit the growth of test microorganisms varied to some extent. These indicated that antimicrobial compounds of bacterial isolates differed. Three bacterial isolates S2T16-1, S3T32-3, and S3T33-3 were selected for further study. The study revealed that methanol extract of bacterial culture inhibited more test microorganisms compared to that of ethyl acetate and n-hexane extract. Among the three isolates, S3T32-3 was the most active one against the test microorganisms.

Key words: *antimicrobial* activity, *Escherichia coli, Candida albicans, Fusarium oxysporum, Ganoderma boninense, Staphylococcus aureus*

INTRODUCTION

Microbial communities are among the most complex, diverse and important groups of organisms in the biosphere. They participate in various biological activities. Microorganisms have been isolated from composts, decaying vegetable matter, lake mud, and other miscellaneous materials [1]. Accordingly, they are an important screening target for a variety of bioactive compounds such as secondary metabolites [2]. Microbial secondary metabolites include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides, antitumor agents and growth promoters of animals and plants [3]. Secondary metabolism is brought on by exhaustion of a nutrient, biosynthesis or addition of an inducer, and/or by a growth rate decrease [3].

Control of unwanted microorganisms is essential in all aspects of life, and microbial diseases must be treated in humans, animals, and plants [4, 5]. However, this lead to rapidly emerged and developed antibiotic resistant microorganism, mainly due to antibiotic misuse [1, 6]. Multidrug resistance is now recognized as a global health problem [1]. Recent solutions involve development of a more rational approach to antibiotic utilization of novel antimicrobial compounds [1, 4].

Most of antimicrobial compounds used in the past several decades were originally isolated from microbial sources [7]. New antimicrobial chemical matter can be discovered in a variety of ways, among which is to screen microorganisms from soil and other natural habitats [1] and large libraries of small molecular mass chemical compounds [7]. Some microorganisms were tested for this purpose. Most investigators appear to employ a wide variety of organisms: Gram-positive and Gram-negative bacteria, fungi pathogenic to man, and fungi saprophytic or parasitic to plants [5, 8, 9].

Several study of isolation and examination of antimicrobial activity of bacteria isolated from agriculture and forest soils in Indonesia have been done, especially of actinomycete group

Bulletin of Environment, Pharmacology and Life Sciences

[10, 11]. The aim of this study was to know antimicrobial activity of bacteria isolated from soil of Sibolangit Natural Recreational Park of North Sumatra, Indonesia. This park is a natural forest with little human disruption. To our knowledge, isolation of bacterial isolates producing antimicrobial compound from this area has not been reported.

MATERIALS AND METHODS

Isolation and Screening of Antimicrobial Producing Bacteria from Soil

Screening for antimicrobial producing bacteria was carried out by spreading 1 ml suspension of 1 g soil samples in 10 ml of sterile distilled water on nutrients agar (NA). Culture was incubated for 1-4 days at ±28-30°C. Bacterial colony growing on agar was carefully observed. The potential bacterial isolates were indicated by clear zone around the colony to inhibit other growing microorganisms. The colony was isolated and sub-culture in NA until pure isolates were obtained.

Pure isolates were subjected to preliminary antimicrobial test to *Escherichia coli* and *Candida albicans*. Paper disc (Oxoid) dropped with 10-µl of 1-day old bacterial isolate culture was placed on *E. coli* and *C. albicans* lawn on Muller Hinton Agar (MHA). The culture was incubated at ±28-30°C for 1 day. Isolate which potential to inhibit the test microbes was observed as clear zone around paper disc.

Examination of Cell Morphology and Biochemical Properties

Cell shape and Gram staining were evaluated using a microscope following standard staining protocols, while colony shape on growing media was observed directly. For motility, each isolate was spot-inoculated on semi-solid medium sulfide indole motility. The diffusion of colony was observed after 24 h of incubation at ±28-30°C. Biochemical properties were examined including catalase test using 3% H_2O_2 solution, gelatin test using gelatin nutrient medium, sugar catabolism test using Triple Sugar Iron Agar, citrate test using Simons Citrate Agar following standard protocol of biochemical test.

Methanol, Ethyl Acetate, and *n*-Hexane Extraction of Antimicrobial Compounds from Potential Bacterial Isolate Cell Culture

Potential bacterial isolates were spread on MHA and incubated for 5 days at ±28-30°C. MHA with the bacterial lawn were then cut into small pieces and put in methanol in dark bottle. The bottle was macerated in dark room for 3 days. Gently shaking was conducted every day during maceration. Solution was filtered and was subjected to be centrifuged at 1.000 g for 15 minutes. Crude extract of filtered solution was heated at 50±2°C to evaporate the solvent using rotary-evaporator until solid extract was produced as previously described by [12] with modification. The extract was put on dark vial and kept in dessicator until used. The same work was also conducted for both ethyl acetate and *n*-hexane extraction.

Antimicrobial Activity Assay of Bacterial Culture Cell Extract

Antimicrobial activity assay was carried out to bacteria *E. coli* and *Staphylococcus aureus*, and fungi *C. albicans, Fusarium oxysporum* and *Ganoderma boninense* using disc diffusion method. For bacterial test, paper disc (Oxoid) of 10-µl of each crude extract of appropriate concentration (40, 60, 80, and 100%) was place on bacterial lawn on MHA. A-10% of dimethyl sulfoxide (DMSO) was used for dissolving the extract. For fungal test, paper disc containing the extracts were put after 4 days of incubation of the fungi on PDA. (-) control was 10% DMSO, and (+) control were ketoconazole for fungi and chloramphenicol (Oxoid) for bacteria, respectively. Cultures were incubated for 1 day at 37°C for bacteria and 2 days at 30°C for fungi, respectively. Inhibitory activity to the bacteria was determined as diameter of clear zone around the disc subtracted with diameter of the disc, while inhibitory activity to the fungi was measured as radius of uninhibited mycellia substracted by radius of inhibited mycelia by the extract.

RESULTS

Characterization of Bacterial Isolates Producing Antimicrobial Compound

Twenty-nine bacterial isolates indicating to exhibit inhibition to the growth of other microorganisms were collected. The ability of these bacteria to inhibit other microbial growth was detected by an inhibition zone around their colonies (Figure 1). Seventeen isolates belonged to Gram-negative and 12 isolates were Gram-positive (Table 1.). Some isolates shared same morphological and biochemical properties, indicating the same bacterial species.

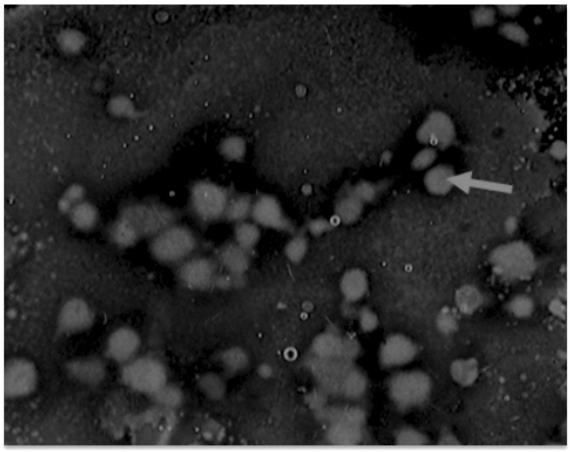

Figure 1. Clear zone around bacterial colony (white arrow) showing inhibition of other growing microorganism

Table 1. Characterization of morphology and biochemistry of bacterial isolates showing to inhibit other microbial colony of soil isolation

Bacterial isolates	Colony characterization	Gram	Cell shape	TSIA					Catalase	Motility	Citrate
				Glucose	Sucrose	Lactose	Sediment	Splinter			
S1T5-1	Entire, flat, white	-	Rod	+	-	-	-	-	+	+	+
S1T5-2	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	+
S1T7-1	Irregular, flat, white	+	Coccus	+	+	+	-	-	+	+	-
S1T8-2	Irregular, flat, white	+	Coccus	+	-	-	-	-	+	+	+
S1T9-1	Irregular, flat, white	-	Coccus	+	-	-	-	-	+	+	+
S1T9-2	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	-
S1T9-3	Irregular, flat, white	-	Coccus	+	-	-	-	-	+	+	-
S1T10-1	Irregular, flat, white	+	Coccus	+	-	-	-	-	+	+	+
S2T1-2	Entire, flat, white	+	Coccus	+	-	-	-	-	+	+	-
S2T16-1	Entire, flat, yellow	-	Coccus	-	-	-	-	-	+	+	+
S2T16-2	Entire, flat, transparent	+	Coccus	+	-	-	-	-	+	+	+
S2T17-1	Entire, flat, transparent	-	Coccus	-	-	-	-	-	+	+	+
S2T17-2	Irregular, flat, white	+	Coccus	+	-	-	-	-	+	+	-
S2T18-2	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	+
S2T21-2	Irregular, flat, white	+	Coccus	+	-	-	-	-	+	+	-
S2T22-1	Irregular, flat, white	-	Coccus	+	-	-	-	-	+	+	+
S2T22-2	Entire, convex, white	-	Coccus	+	-	-	-	-	+	+	-
S2T23-1	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	+
S2T25-1	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	-
S3T26-1	Irregular, flat, white	-	Coccus	+	-	-	-	-	+	+	-
S3T26-2	Irregular, flat, white	-	Coccus	+	-	-	-	-	+	+	+
S3T27-1	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	-
S3T31-2	Entire, convex, white	+	Coccus	+	+	+	-	-	+	+	-
S3T32-1	Irregular, flat, white	+	Coccus	+	-	-	-	-	+	+	-
S3T32-3	Entire, flat, white	-	Coccus	+	-	-	-	-	+	+	-
S3T32-4	Irregular, flat, white	+	Coccus	+	-	-	-	-	+	+	-
S3T33-1	Entire, flat, white	+	Coccus	+	-	-	-	-	+	+	-
S3T33-2	Entire, flat, white	+	Rod	+	-	-	-	-	+	+	-
S3T33-3	Irregular, flat, white	-	Coccus	+	-	-	-	-	+	+	-

Many groups of microorganisms such as Gram-positive, Gram-negative bacteria and fungi have the ability to synthesize antimicrobial compounds such as antibiotics, enzymes, antifungal proteins, or other antimicrobial compounds to inhibit the growth of other microorganisms [13]. The prominent

antimicrobial agent producers present in soils are the actinomycetes [9, 14]. However, this study did not refer to actinomycetes in particular. Secondary metabolite is to become an important use in controlling various diseases in plants, animals and humans. The process of production usually involves screening of wide range of microorganisms, testing and modification [1].

Assay of Antimicrobial Activity of Bacterial Isolates

Potential bacteria isolated from soil samples were performed to inhibit the growth of test microorganisms *E. coli* and *C. albicans* representing bacteria and fungi. The result showed that the ability to inhibit the growth of test microorganisms varied (Figure 2). Some isolates showed to suppress only bacterial growth, while other isolates inhibited fungal growth. Among them, S3T33-3 was more active to inhibit the growth of *E. coli*, while S2T16-1 and S2T16-1 were more active on *C. albicans* growth (Table 2). This indicated that antimicrobial compound and its number produced by such isolate differently. Few bacteria such as *Streptomyces coelicolor* [15, 16] and *Pantoea agglomerans* [17] produce more than one antibiotic.

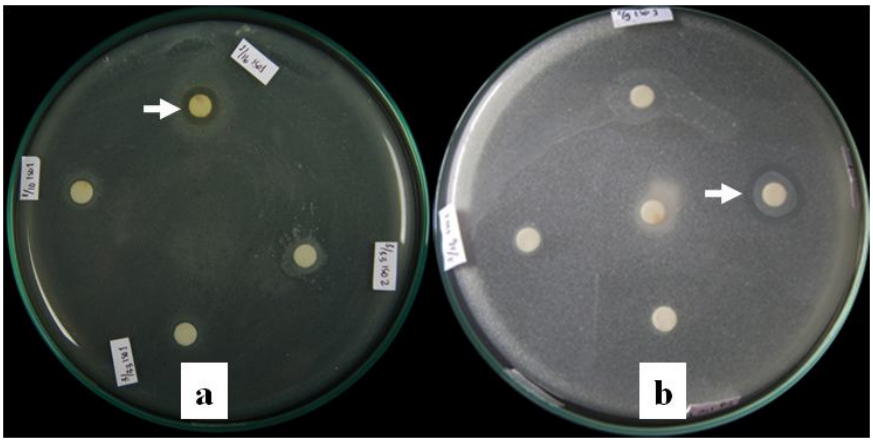

Figure 2. Antimicrobial activity of the bacterial isolates to (A) *E. coli* and (B) *C. albicans*. White arrow is inhibition zone of the bacterial isolates in the lawn

Table 2. Antagonistic effect indication of the bacterial isolates to *E. coli* and *C. albicans*

Bacterial isolates	Inhibition zone		Bacterial isolates	Inhibition zone	
	E. coli	*C. albicans*		*E. coli*	*C. albicans*
S1T5-1	No	Yes	S2T22-1	No	No
S1T5-2	No	No	S2T22-2	No	No
S1T7-1	No	No	S2T23-1	No	No
S1T8-2	No	Yes	S2T25-1	Yes	No
S1T9-1	Yes	No	S3T26-1	No	No
S1T9-2	No	No	S3T26-2	No	No
S1T9-3	Yes	No	S3T27-1	No	Yes
S1T10-1	No	Yes	S3T31-2	No	No
S2T1-2	No	No	S3T32-1	No	Yes
S2T16-1	No	Yes	S3T32-3	No	Yes
S2T16-2	Yes	No	S3T32-4	No	No
S2T17-1	No	No	S3T33-1	No	Yes
S2T17-2	No	No	S3T33-2	No	No
S2T18-2	No	No	S3T33-3	Yes	No
S2T21-2	No	No			

Antimicrobial Assay of Methanol, Ethyl acetate, and *n*-hexane Extracts of Selected Bacterial Isolates

Three isolates, S2T16-1, S3T32-3, and S3T33-3 were chosen for further study since they showed more potential to inhibit test microorganisms by producing higher inhibition zone either on *E. coli* or *C. albicans* lawn. Cell culture of these isolates was extracted using methanol, ethyl acetate, and *n*-hexane. Depending upon type of antimicrobial compounds released and its concentration in the extract, the ability to inhibit microbial growth of each extract was different (Figure 3). Assay of antimicrobial activity of all extracts showed that more extracts inhibited the growth of Gram-negative *E. coli* and fungi compared to that of Gram-positive *S. aureus* (Table 3.). The antimicrobial assay of the extract of selected bacterial isolates is presented in Table 3.

Methanol extract of S2T16-1 and S3T32-3 showed no ability in inhibiting all test bacteria. However, it displayed antifungal activity, while methanol extract of S3T33-3 showed the ability to inhibit all test microorganisms. No inhibition of all test microorganisms was shown by ethyl acetate extract of S2T16-1, except that of *G. boninense*, while ethyl acetate extract of S3T32-3 inhibited the growth of *E. coli*, *F. oxysporum* and *G. boninense*. On the other hand, only microbial growth of *G. boninense* was inhibited by ethyl acetate extract of S3T33-3. All *n*-hexane extract of bacterial isolates did not show inhibition to test microorganisms as well but that of S3T33-3 which demonstrated to inhibit *E. coli*, *F. oxysporum* and *G. boninense*, and that of S3T32-3 was active againts *G. boninense*.

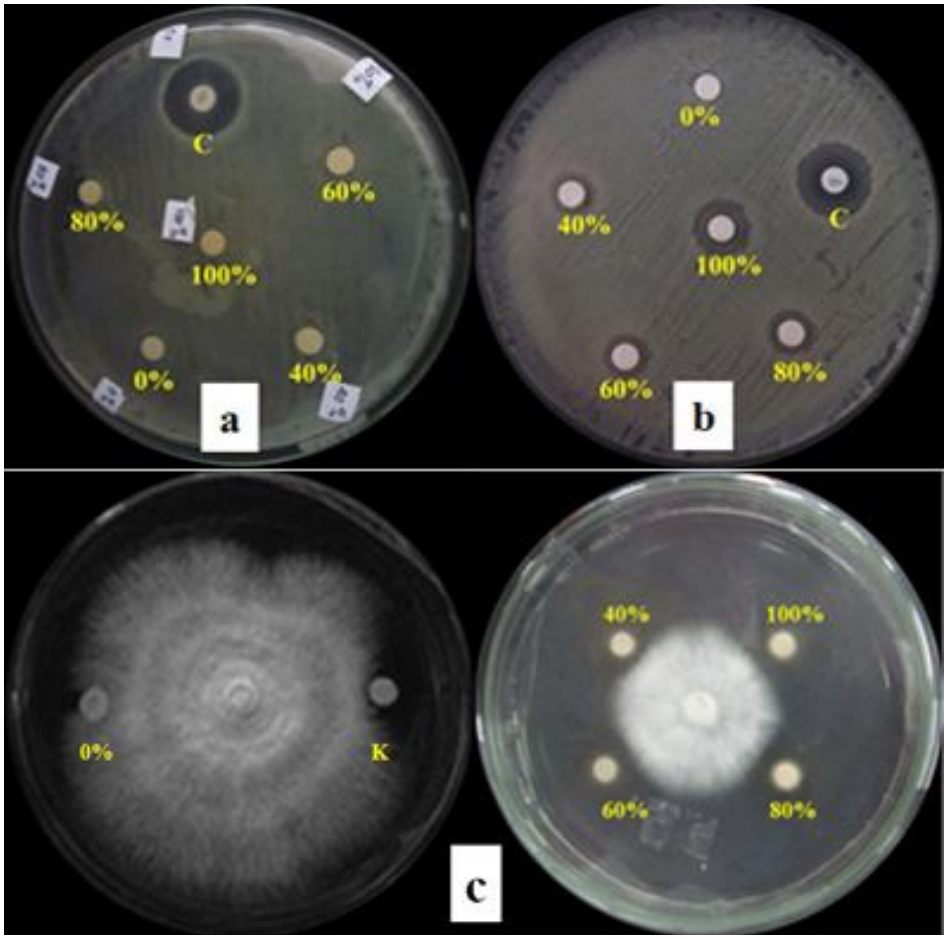

Figure 3. Antimicrobial assay of methanol extract of S2T16-1 to (a) *E. coli* (no inhibition showed), S3T33-3 to (b) *S. aureus*, and S2T16-1 to (c) *G. boninense*. (C: chloramphenicol, K: ketoconazole)

Table 3. Antimicrobial assay of methanol, ethyl acetate, and *n*-hexane extracts of selected bacterial isolates to bacteria *E. coli* and *S. aureus*, and fungi *C. albicans*, *F. oxysporum* and *G. boninense*

Test microorganisms	Extract concentration (%)	Extract inhibition zone (mm)								
		Methanol			Ethyl acetate			*n*-heksane		
		S2T16-1	S3T32-3	S3T33-3	S2T16-1	S3T32-3	S3T33-3	S2T16-1	S3T32-3	S3T33-3
E. coli	0	0	0	0	0	0	0	0	0	0
	40	0	0	13.03	0	15.30	0	0	0	6.70
	60	0	0	15.46	0	15.56	0	0	0	13.50
	80	0	0	17.26	0	18.63	0	0	0	9.70
	100	0	0	19.73	0	21.93	0	0	0	18.83
S. aureus	0	0	0	0	0	0	0	0	0	0
	40	0	0	11.46	0	0	0	0	0	0
	60	0	0	11.43	0	0	0	0	0	0
	80	0	0	11.80	0	0	0	0	0	0
	100	0	0	14.83	0	0	0	0	0	0
C. albicans	0	0	0	0	0	0	0	0	0	0
	40	6.33	16.63	13.20	0	0	0	0	0	0
	60	8.93	19.20	14.53	0	0	0	0	0	0
	80	6.07	18.90	16.58	0	0	0	0	0	0
	100	10.00	19.20	17.13	0	0	0	0	0	0
F. oxysoporum	0	0	0	0	0	0	0	0	0	0
	40	0.83	1.08	1.23	0	0.33	0	0	0	0
	60	0.69	1.58	0.04	0	0.67	0	0	0	0
	80	0.79	1.42	0.99	0	0.75	0	0	0	0.50
	100	1.53	2.22	1.55	0	2.08	0	0	0	1.00
G. boninense	0	0	0	0	0	0	0	0	0	0
	40	6.90	7.23	5.18	2.24	7.41	5.60	0	7.16	6.32
	60	7.73	6.47	9.05	2.38	8.01	4.00	0	6.96	4.87
	80	7.90	9.40	7.68	4.84	8.61	3.46	0	6.79	5.75
	100	9.40	10.97	7.18	6.77	8.71	6.07	0	6.26	7.85

DISCUSSION

Variations in the degree of activity of different antagonistic cultures are well known [13]. Effectiveness of antimicrobial chemical compound was affected by permeability, specificity for the target, and effect of the drug on the host impact [7]. The ability to inhibit the growth of other microorganisms also depend on whether the antimicrobial compounds are excreted out of cells or accumulated in the cell [12]. Bacterial isolates may have a gene coding for the formation of the metabolites, but no expression in normal circumstances [3]. The isolates may produce antimicrobial compounds, but is not active against test microorganisms, or produced different antimicrobial spectrum [12]. Antibiotics may show weak inhibition since some isolates produce them in small quantities. However, in high concentration these antibiotics may possess new and useful properties.

The use of high concentration of the extract to inhibit test microorganisms in this study indicated that in the growth medium the isolates might only produce small quantity of the antimicrobial compounds. The medium used for cross streak or other tests for activity is also important, since antibiotics may not be optimally produced in improper medium [18]. Many studies were to find media and optimum environmental condition for optimization of antibiotic production [1, 5, 18, 19]. For example, the production of antibacterial antibiotic by *Streptomyces kanamyceticus* M 27 showed to optimize with dextrose and $(NH_4)H_2PO_4$ as carbon and nitrogen source, respectively in

alkaline pH [18]. Solubility of the metabolites produced in the solvent used also influence the differences in this inhibition. In general methanol extract showed more inhibition to different test microorganisms. Methanol is known to pull out more substances from extracted materials rather than ethyl acetate and *n*-hexane which are semi-polar and non-polar, respectively.

ACKNOWLEDGEMENTS

We would like to thank to DITLITABMAS of Directorate General of Higher Education, Indonesia Ministry of Education and Culture for supporting this research.

REFERENCES

1. Muhammad, S.A., Ahmad, S. & Hameed, A. (2009). Antibiotic production by thermophilic *Bacillus specie* Sat-4. *Pak. J. Pharm. Sci.* 22: 339-345.
2. Hackl, E., Boltenstern, S., Bodrossy, L. & Sessitsch, A. (2004). Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Appl. Environ. Microbiol.* 7: 5057-5065. DOI:10.1128/AEM.70.9.5057-5065.2004.
3. Demain, A.L. (1998). Induction of microbial secondary metabolism. Review Article. *Int. Microbiol.* 1: 259-264.
4. Gram, L., Melchiorsen, J. & Bruhn, J.B. (2010). Antibacterial activity of marine culturable bacteria collected from a global sampling of ocean surface waters and surface swabs of marine organisms. *Mar. Biotechnol.* 12: 439-451. DOI 10.1007/s10126-009-9233-y.
5. Nanjwade, B.K., Chandrashekhara, S., Shamarez, A.M., Goudanavar, P.S. & Manvi, F.V. (2010). Isolation and morphological characterization of antibiotic producing Actinomycetes. *Trop. J. Pharmaceut. Res.* 9: 231-236.
6. Uzair, B., Ahmeda, N., Mohammad, F.V., Ahmad, V.U. & Edwards D. 2009. Screening of marine bacteria of Pakistan coast for drug discovery potential. *Proc. Pakistan Acad. Sci.* 46:137-144.
7. Pathania, R. & Brown, E.D. (2008). Small and lethal: Searching for new antibacterial compounds with novel modes of action. Minireview. *Biochem. Cell Biol.* 86: 111-115. DOI: 10.1139/O08-011.
8. Petatán-Sagahón, I., Anducho-Reyes, M.A., Silva-Rojas, H.V., Arana-Cuenca, A., Tellez-Jurado, A., Cárdenas-Álvarez, I.O. & Mercado-Flores, Y. (2011). Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora. Int. J. Mol. Sci.* 12: 5522-5537. DOI:10.3390/ijms12095522.
9. Ceylan, O., Okmen, G. & Ugur, A. (2008). Isolation of soil *Streptomyces* as source antibiotics active against antibiotic-resistant bacteria. *EurAsia J. BioSci.* 2: 73-82.
10. Susilowati, D.N., Hastuti, R.D. & Yuniarti, E. (2007). Isolasi dan Karakterisasi Aktinomisetes Penghasil Antibakteri Enteropatogen *Escherichia coli* K1.1, *Pseudomonas pseudomallei* 02 05, dan *Listeria monocytogenes* 5407. *J. AgroBiogen.* 3: 15-23.
11. Dharmawan, I.W.E., Kawuri, R. & Parwanayoni, M.S. (2009). Isolasi *Streptomyces spp.* pada kawasan hutan provinsi bali serta uji daya hambatnya terhadap lima strain diarrheagenic *Escherichia coli* (Isolation of *Streptomyces spp.* from several forest areas in Bali and their inhibition activities to five strains of diarrheagenic *Escherichia coli*). *J. Biol.* 13: 1-6.
12. Nofiani, R., Nurbetty, S. & Sapar, A. (2009). Aktivitas antimikroba ekstrak metanol bakteri berasosiasi dengan spons dari Pulau Lemukutan Kalimantan Barat. (Antimicrobial activities of methanol extract from unidentified sponge associated bacteria in Lemukutan Island, Kalimantan Barat). *E-Jurnal Ilmu dan Teknologi Kelautan Tropis* 1: 33-41.
13. Pandey, B., Ghimire, P., Prasad, V. & Agrawal, V. (2002). *Studies of the antimicrobial activity of the actinomycetes isolated from the Khumby region of Nepal*. Department of Bacteriology. University of Wisconsin- Madison. Madison. Wisconsin.
14. Baltz, R.H. (2005). Antibiotic discovery from actinomycestes: Will a renaissance follow the decline and fall? *SIM News* 58: 186-196.
15. Price, B., Adamidis, T., Kong, R. & Champness, W. (1999). *Streptomyces coelicolor* antibiotic regulatory gene, *absB*, encodes an RNase III homolog. *J. Bacteriol.* 181: 6142–6151.
16. Nodwell, J.R. (2007). Novel links between antibiotic resistance and antibiotic production. Guest commentary. *J. Bacteriol.* 189: 3683–3685. DOI:10.1128/JB.00356-07.
17. Wright, S.A., Zumoff, C.H., Schneider, L. & Beer, S.V. (2001). *Pantoea agglomerans* strain Eh318 produces two antibiotics that inhibit *Erwinia amylovora in vitro*. *Appl. Environ. Microbiol.* 67: 284-292. DOI:10.1128/AEM.67.1.284-292.2001.
18. Nanjwade, B.K., Chandrashekhara, S., Goudanavar, P.S., Shamarez, A.M. & Manvi FV. (2010). Production of antibiotics from soil-isolated Actinomycetes and evaluation of their antimicrobial activities. *Trop. J. Pharmaceut. Res.* 9: 373-377.
19. Oskay, M. (2009). Antifungal and antibacterial compounds from *Streptomyces* strains. *Afr. J. Biotechnol.* 8: 3007-3017.