BIOPROSPECTING SOIL ACTINOMYCETES: ISOLATION AND ANTIBACTERIAL ASSAY

(Bioprospek Aktinomiset Tanah: Isolasi dan Uji Antibacteri)

Suhartono[1], Risa Nursanty[2]
[1,2]Biology Department, Faculty of Mathematics and Natural Sciences
Syiah Kuala University
suhartono@fmipa.unsyiah.ac.id

Abstract

Searching fornovel bioactivecompounds of microorganismsis increasing. Actinomycetesare a groupof bacteria thathave potential asa majorproducerof bioactivecompounds. This study aimedtoisolateand evaluate theisolates of actinomycetes collected fromsoil. Thisresearch was carried out in threestages, namely the field sampling, isolation ofactinomycetes, screening the potentialas a producer ofbioactivecompounds(antimicrobials). A total of11 isolates ofactinomyceteshave been isolatedin this study. The isolates varied in morphological characteristics. The antibacterial evaluation showed that nine isolates (81.8%) that were able to inhibit either the test organism. Three isolates of ACT-04, ACT-05, ACT-06 and ACT-11 were capable of inhibiting both bacterial targets.
Key words: *Bioprospecting, soil, actinomycetes*

Abstrak

Pencarian senyawabioaktifbarudarimikroorganismesemakin meningkat. Aktinomyset adalahkelompokbakteri yang memilikipotensisebagaiprodusenutamasenyawabioaktif.Penelitianinibertujuanuntukmengisolasida nmenguji isolataktinomisetes yang diisolasi daritanah.Penelitianinidilakukandalamtigatahap, yaitupengambilansampellapangan, isolasiaktinomisetes, dan penapisan potensisebagaipenghasilsenyawabioaktif (antimikroba).Sebanyak 11 isolataktinomisetestelahberhasil diisolasidalampenelitianini.Isolatmemilki karakteristikmorfologi yang beragam.Sembilanisolat (81,8%) diketahui mampumenghambatbakteri target. Tigaisolat ACT-04, ACT-05, ACT-06 dan ACT-11 mampumenghambat target keduabakteri.
Kata Kunci: *biosprospek, tanah, aktinomiset*

INTRODUCTION

Searching fornovel bioactivecompoundsfrom microorganism is increasing.This is as a result of thedecreaseof effectivenessandefficiencyfor the drugs administered to deal withinfectious diseases. The decline wascaused by theresistanceof thepathogentriggeredby inappropriate use of the drugs. Actinomycetes are filamentous Gram-positive bacteria with true aerial hyphae and high G/C content belonging to the phylum Actinobateria (order actinomycetales) (Madigan *et al*. 2010). This group of bacteria are primary found in soil(Nurjasmi *et al.*, 2009; Deepa *et al*., 2011; George *et al.*, 2012; Istianto *et al*, 2012) and water (Radhika*et al*, 2011; Gulve and Deshmukh, 2012), but some of them are endophytic in plants (Shimizu, 2011).

The vast majority of actinomycetes are producers of biologically active compounds, such as antibacterial, antiviral, and antifungal agents. Of all the practically used antibiotics more than 90% originate from actinomycetes, and about two-thirds of all the discovered bioactive substances of microbial origin are produced by this group of bacteria (Hamaki *et al*., 2005).Actinomyctes, particularly from the genus of *Streptomyces*, has been widely known as antibiotic producers, such as streptomycinproduced by*Streptomycesgriseus*, erythromycinproduced by*Streptomyceserythrus*, chloramphenicolproducedby*Streptomycesvenezuelae*andtetracyclineproduced by*Streptomycesaureofaciens*(Hopwood, 2007).

The research objective was to isolate actinomycetes from soil samples as well as to evaluate the isolates for their bioactive compounds showing antibacterial activity.

METHOD

Materials used in the research comprised soil, culture of *Escherichia coli*ATCC 25922 and *Staphyloccusaureus*ATCC 29213, alkohol 70%, *yeast malt extract* (YM) media, *nutrient agar* (NA) media, *nutrient broth* (NB) media, nystatin,and aquadest. The equipment used in the research consisted of plates, micropipette, tips,cork borer, incubator, shaker, and other general microbiology equipment.

Sample collection and pre-treatment

Soil samples were collected from three locations in the campus of Syiah Kuala university. The locations were Faculty of Mathematics and Natural Sciences, Faculty of Veterinary Sciences, and Faculty ofEnginering. The soil samples were taken fromthe 20 cm in depth after removing upper soil surface. The samples wereplaced in the sterile jar, closed tightly andstored in a refrigerator. The physical feature of the sample locations (temperature, acidity and the location coordinate) were also measured in this research. The samples were then pre-treated by heating in oven at 60°C for two days before they were used in the isolation step.

Isolation of Actinomycetes

A total of 1 gram of sample was added into reaction tube containing 9 ml of sterile aquadest. The sample was then serially diluted until the suspension reached $10^{-3}$of dilution. A 0,1 ml of suspension was equally spreaded onto petri disks containing YM agar media (in g/l: *yeast extract* 4, *malt extract* 10, glukosa 4, dan*bacto* agar 15) pH 7.2 and nystatin. The petri disks made in duplo were then incubated at 28°C for 7-14 days.The growing actinomycetes were then purified by streaking into fresh YM media before they were observed and characterized for their morphological features.

Screening and Antibacterial Assay

All the actinomycetes isolates collected in the isolation step were screened for their antibacterial activity againts test organisms by using an antagonistic assay. The test organism consisted of *E.coli* and *S.aures* as representation of gram negative and gram prositive bacteria respectively. Briefly, a 0.1 ml of test organisms was evenly inoculated onto plates containing NA. By using a cork borer (5mm in diameter) actinomycete isolates were place into the plates before they were incubated at room temperature for 48 hours. A clear zone formed surrounding the isolates was measured as an indication of antibacterial activity.

RESULT AND DISCUSSION

Actinomycetes Isolation

There were 11 Actinomycetes isolates that has been succesfullyrecovered in the research. The isolates are coded as ACT-01, ACT-02, ACT-03, ACT-04, ACT-05, ACT-06, ACT-07, ACT-08, ACT-09, ACT-10, and ACT-11. All isolates harboured various morphological appereance as tabulated in Table 1 and shown in Figure 1.

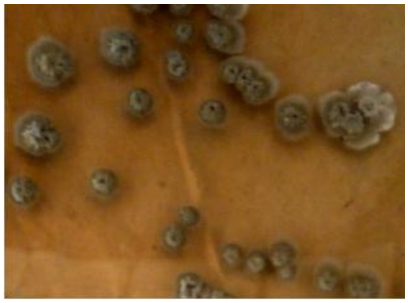
Thecharacteristics ofactinomycetescolonies generally possesess circular shape with round margin, andraised elevation. Moreover, the the myceliumof the actinomycetes isolates show various colour. Actinomyceteshave twokinds ofmycelium, aerial myceliumthat is(surface) and thesubstratemycelium(vegetative). Boththe myceliumis capable of producingthe pigment thatcauses thecolor differencein eachcolonyaccording tothe typeactinomycetes. Of thetotal11isolates, there are 10colonies ofactinomycetesmycelium showing variouscolors, namely, the gray(10%), cream(45.4%), white(18.1%), chocolate(10%), andorange(18, 1%). One ofthe main characteristicsofactinomycetesthat is thepigmentproduced by themycelium, whichdependon the composition ofmediaused and thecultivationconditionsapplied(Kampfer, 2006). The resultingpigmentmelaninis usually acompoundis the key factorin theidentification of theactinomycetes(Arai andMikami, 1972). In addition, thepresence ofpigmentationcan also be usedas a basis forthegrouping ofsomeisolates ofactinomycetes that were recovered(Frewari, 1999;Widuretno, 2000;Desrianietal., 2004).

Screening and Antibacterial Assay

A total of 11 isolates obtained in this study was examined for their potential as a producer of antibacterial compounds against test bacteria, namely *Escherichia coli* and *Staphylococcus aureus*. Test results of actinomycetesantagonistic assay against test bacteria are presented in in Figure 2.

Table 1. Morphological features of Actinomycetes isolates

No	Isolate Code	Colony Morphological Characteristics			
		Color	**Shape**	**Margin**	**Elevation**
1.	ACT-01	Grey	Circular	Undulate	Crateriform
2.	ACT-02	White	Concentric	Filiform	Raised
3.	ACT-03	Cream	Circular	Filamentous	Raised
4.	ACT-04	Cream	Circular	Entire	Pulvinate
5.	ACT-05	Brownish Grey	Contoured	Entire	Convex
6.	ACT-06	Chocolate	Circular	Entire	Crateriform
7.	ACT-07	Creamish white	Circular	Filementous	Umbonate
8.	ACT-08	Orange	Irregular	Undulate	Raised
9.	ACT-09	Whitish orange	Wrinkled	Undulate	Raised
10.	ACT-10	Cream	Irregular	Irregular	Raised
11.	ACT-11	Whitish Cream	Irregular	Undulate	Raised

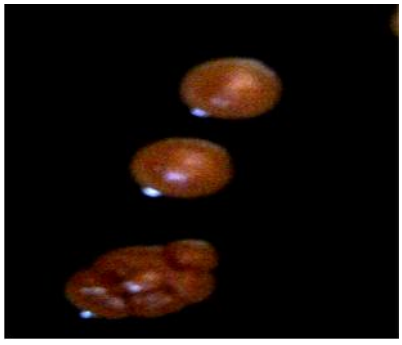


(a)

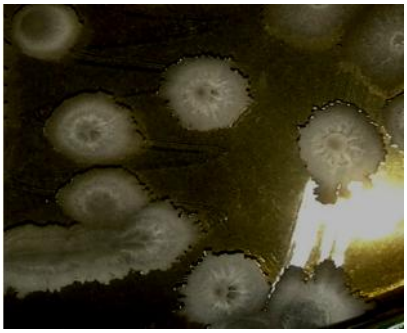
(b)

(c)

(d)



(e) (f)



(g) (h)

(i) (j)

(k)

Figure 1. ActinomycetesIsolates (a) ACT-01; (b) ACT-02; (c) ACT-03; (d) ACT-04; (e) ACT-05; (f) ACT-06; (g)ACT-07; (h) ACT-08; (i) ACT-09; (j) ACT-10; and (k) ACT-01.

There were nineisolates(81.8%) that were able to inhibiteitherthe targetbacteria. Isolates of ACT-04, ACT-05, ACT-06 andACT-11 were capable ofinhibitingbothbacterialtargets. The diameter ofthe clear zoneformedin inhibiting*E. coli* ineach oftheseisolatesis2.5mm, 0.6mm, 6 mmand0.6mm, respectively. Against *S. Aureus*, theses four isolates formedinhibitory zone 5.2mm,5.7mm, 5 mmand5 mm in diameter. Variety in the amount ofinhibitoryzonesobtained inthe studydue to thesecondarymetabolitesproducedeachisolate conferring different chemical structure, compositionandconcentration (Hopwood, 2007; Jawetz *et al.* 2010). George *et al*. (2012) has succesfully isolated 74 strains of actinomycetes from different soil samples which more than 75% of them showed biological activities in supressing in different degrees the growth of test pathogens.

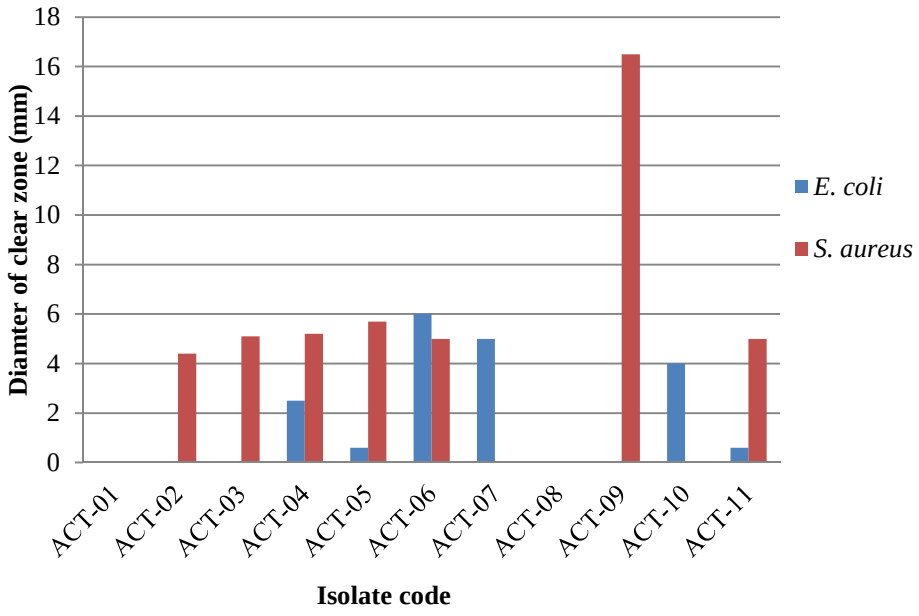


Figure 2. Antibacterial assay of Actinomycetes isolates

The ability of actinomycetes to inhibit the growth of other bacteria due to the release of extracellular metabolites. This is indicated by the presence of clear zone around the actinomycetes isolates. Naturally, secondary metabolites produced by actinomycetes can serve as antimicrobials to compete with others in terms of microbial nutrients (Madigan *et al.*, 2010). Bioactive compounds that could inhibit other microorganisms may be derived from the fungi and bacteria, both gram positive and negative (Waksman *et al.*, 2010) due to the ability of bacteriostatic and bacteriolytic effects (Welsch, 1942).

Twoisolates ofactinomycetes(18.1%) of thetotal11isolatesobtainedshowed noinhibitoryactivityagainsttest bacteria. However, they might be capable of inhibiting any other test bacteria or organims.

CONCLUSION

As many as 11 isolates have been successfully recovered from the soil samples. The isolates varied in morphological characteristics. The antibacterial evaluation showed that nineisolates(81.8%) that were able to inhibiteither the testorganism. Three isolates of ACT-04, ACT-05, ACT-06 andACT-11 were capable ofinhibitingbothbacterialtargets.

AKNOWLEDGEMENT

Authors are thankful to the I-MHERE project of Syiah Kuala University (contract No. 002/RG/HEI-IU I-MHERE/2010) that has granted this research. Also, authors aknowledge to Sri Mulyani who supported this research.

REFERENCES

Deepa, LS., Gowthami, K, Kumar, KS. 2001. In vivo Screening of Antimicrobial Activity of Soil Actinomycetes Against Human Pathogens. *World J. Agric. Sci* 7 (5): 624-628.

Desriani, Lestari, Y., Meryandini, A. 2004. Penapisanisolat*Streptomyces*spppe nghasil protein penghambat β-Laktamase.*Hayati* 11(3): 88-92.

Frewari, I. 1999. Penapisanbiologisdanisolasisenya wapeptidaantimikrobdari*Streptomy ces* sp.. [skripsi]. Bogor: FakultasMatematikadanIlmuPenge tahuanAlam, InstitutPertanian Bogor

George, M., Anjumol, A., George, G., Hatha, A.A.M. 2012. Distribution and bioactive potential of soil actinomycetes from different

ecological habitats. *Afr. J. Microbiol. Res.* 6(10): 2265-2271.

Gulve, R.M., Deshmukh, A.M., 2012. Antimicrobial activity of the marine actinomycetes. *Int Multidis Res J*. 2(3):16-22.

Hamaki T, Suzuki M, Fudou R, Jojima Y, Kajiura T, Tabuchi A, Sen K, Shibai H. 2005. Isolation of novel bacteria and actinomycetes using soil-extract agar medium. *J Biosci Bioeng*, 99: 485–492.

Hopwood, D.A. 2007. *Streptomyces in Nature and Medicine the Antibiotic Makers*. New York: Oxford University Press.

Istianto, Y., Koesoemowidodo, R.S.A., Saputra, H., Watanabe, Y., Pranamuda, H., Marwoto, B. 2012. Application of Phenol Pretreatment for the Isolation of Rare Actinomycetes from Indonesian Soil. *Microbiol Indones*. 6(1):42-47.

Jawetz, E. Melnick, J.L., Adelberg, E.A., Geo, F.B., Karen, C.C., Janet, S.B., Stephen, A.M., and Timothy, A.M. 2010. *Medical Microbiology* 25th Ed. The McGraw-Hill Companies, USA.

Madigan, M.T., Martinko, J.M., Stahl, D., Clark, D.P. 2010. *Biology of Microorganism*. Ed ke-13. New York: Benjamin Cummings, Inc.

Nurjasmi, R., Widada, J., Ngadiman. 2009. Diversity of Actinomycetes at Several Forest Types in Wanagama I Yogyakarta and Their Potency as a Producer of Antifungal Compound. *I.J. Biotech.* 14(2): 1196-1205.

Radhika. S., Bharathi1, S., Radhakrishnan, M., Balagurunathan, R. 2011. Bioprospecting of Fresh Water Actinobacteria:Isolation, ntagonistic Potential and Characterization of Selected Isolates. *J. Pharm. Res.* 4(8): 2584-2586.

Shimizu, M. 2011. Endophytic Actinomycetes: Biocontrol Agents and Growth Promoters. D.K. Maheshwari (ed.), Springer-Verlag Berlin Heidelberg.

Widuretno, D. 2000.PenapisanisolatStreptomyces sp. penghasilsenyawapenghambat Escherichia coli resistenampisilin. [skripsi]. Bogor: FakultasMatematikadanIlmuPengetahuanAlam, InstitutPertanian Bogor.

Waksman, S. A., Schatz, A. and Reynolds, D. M. 2010.Production of Antibiotic Subtances by Actinomycetes.*Annals of the New York Academy of Sciences* 1213: 112–124.

Welsch, M. 1942.Bacteriostatic and Bacteriolytic Properties of Actinomycetes.*Journal of Bacteriology* 44(5): 571–588.