KATA PENGANTAR

Dengan penuh rasa syukur kehadirat Allah SWT, Media Farmasi Vol. 12 No. 1 Tahun 2015 telah terbit.

Pada edisi ini, Jurnal Media Farmasi menyajikan 11 artikel yang kesemuanya merupakan hasil penelitian. Enam artikel dari luar Fakultas Farmasi UAD membahas, (1) Formulasi dan evaluasi masker wajah *peel-off* yang mengandung kuersetin (2) Pengaruh polivinil pirolidon (PVP) dalam absorpsi piroksikam (3) Uji perbandingan aktivitas antijamur *Pityrosporum ovale* dari kombinasi ekstrak etanol buah belimbing wuluh dan daun sirih (4) Aktivitas inhibisi α-amilase ekstrak karagenan dan senyawa polifenol (5) Uji antihipertensi infus kombinasi biji dan rambut jagung (6) Layanan pesan singkat pengingat meningkatkan kepatuhan minum obat. Lima artikel dari peneliti Fakultas Farmasi UAD yang membahas tentang : (1) Formulasi emulgel minyak biji bunga matahari (2) Aktivitas antifungi fraksi etil asetat ekstrak daun pacar kuku (3) Karakteristik genetik *Actinomycetes* (4) Simvastatin sebagai hepatoprotektor (5) Faktor yang diprediksi berpengaruh terhadap pengobatan sendiri.

Harapan kami, jurnal ini dapat bermanfaat bagi pembaca atau menjadi referensi peneliti lain. Kritik dan saran membangun, senantiasa kami terima dengan tangan terbuka.

Dewan Editor

GENETIC CHARACTERISTICS OF ACTINOMYCETES ISOLATES (GST, KP, KP11, KP16, T24, T37 CODE) BY RFLP OF NRPS GENES

Melati Aprilliana Ramadhani, Nanik Sulistyani

Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta, Indonesia
Email : la_ela2404@yahoo.co.id

ABSTRACT

Actinomycetes is the one of antibiotic producing microorganisms. Actinomycetes isolates with GST, KP, KP11, KP16, T24, T37 have been isolated from soil. This study aims to determine the diversity of actinomycetes isolates by RFLP (Restriction Fragment Length Polymorphism) of NRPS (Non Ribosomal Peptide Synthetase) genes from GST, KP, KP11, KP16, T24, and T37 isolates. This study is divided into several steps of the DNA isolation from isolate GST, KP16, T24, T37, KP, KP11, PCR of the 16S rRNA and NRPS genes and cutting the NRPS genes fragmens with HaeIII enzyme to determine the diversity of isolates. Results of DNA isolate, PCR of 16S rRNA genes and NRPS genes, and RFLP of NRPS genes performed by agarose gel electrophoresis. The diversity of the isolates were analyzed by multivariate analysis UPGMA with MVSP 2.0 applications. The results showed the diversity of 6 actinomycetes isolates are T37 and GST have 64 % similarity, KP11 and KP16 have 96 % similarity, KP has 76 % similarity with KP11 and KP16, while T24 has 60 % similarity with KP and 12 % similarity with T37 and GST. Based on RFLP analysis of the NRPS genes, among of 6 isolates can divided into 4 groups are T37 and GST isolates as group 1, KP11 and KP16 isolates as group 2, KP isolate as group 3, and T24 isolate as group 4.

Keyword : *Actinomycetes, diversity, 16S rRNA genes, NRPS genes, RFLP*

INTRODUCTION

NRPS (*Nonribosomal peptide synthetase*) is biosynthetic systems involved in the synthesis of a large number of important biologically active compounds produced by microorganisms, among others by actinomycetes (Sacido *et al.*, 2005). NRPS is multi-enzymatic, multi-domain megasynthases involved in the biosynthesis of nonribosomal peptides. These secondary

metabolites exhibit a remarkable array of biological activity and many

of them are clinically valuable anti-microbial, anti-fungal, anti-parasitic, anti-tumor and immunosuppressive agents. Nonribosomal peptides are biosynthesized by sequential condensation of amino acid monomers (Ansari *et al.*, 2004).

NRPS modules contain the activities corresponding to the condensation, adenylation, and thiolation steps involved in the recognition and condensation of the substrate. Additional domains (heterocyclase, N-methylase, epimerase, thioesterase, and reductase) are also present depending on the requirements for the substrate activation, elongation, and modification (Sacido *et al.*, 2005). In the previous decades, natural products screening programs have concentrated an intense effort in the discovery of biologically active metabolites produced by actinomycetes (Sacido *et al.*, 2005).

Genetic diversity of NRPS genes isolates of actinomycetes can be analyzed by the method of *Restriction Fragment Length Polymorphism* (RFLP). RFLP known as cleaved amplified polymorphic sequences, is a simple, reliable, relatively fast, and inexpensive method with minimum equipment requirements. RFLP is based on the creation or deletion of recognition site of a restriction endonuclease by nucleotide variations in the polymorphic site; thus, digestion of the PCR product containing the polymorphism with an appropriate restriction endonuclease results in disparate electrophoretic patterns by polymorphism genotype (Sharafi *et al.*, 2012).

The principle of this method is cutting gene become fragments with restriction enzyme. Differences size of the fragments cutting result, showed of gene diversity are analyzed. In the case, the RFLP of NRPS genes profile, then indicates the existence of differences secondary metabolites are resulting (Faizal *et al.*, 2008).

The objective of this research was to observe the diversity of 6 actinomycetes isolates with GST, KP16, T24, T37, KP, and KP11 code based on the RFLP of NRPS genes profile. The results of the research can be used to identify the diversity

and characterization of actinomycetes isolates.

METHODOLOGY

The tools used in this research are: glass tools, PCR, electrophoresis, UV lamp.

The materials used are the SNB media, agarose 1%, agarose 2%, Etidium bromide, DNA Ladder (Marker), A7R and A3F primary, F27 and R1492 primary, buffer R, enzyme BsuRI.

DNA Isolation

Culture Preparation

Culture preparation has been done by filling 20 ml starter inside an erlenmeyer containing 200 ml medium SNB already sterilized. The media containing 20 ml starter, was incubated for 5 days by stirer with magnetic stirring. The preparation of this culture has been done at LAF room to minimize the occurrence of contamination (Sulistyani *et al*, 2013).

DNA Isolation

DNA isolation method based on Farida *et al.* (2007) with modification. The isolates of actinomycetes of 1-2 ml was taken, and then centrifuged with 5000 rpm 10 minutes. Pellet was washed with 200 µl of buffer TE. Mixed pellets with 50 µl lisosim, incubated at 37^{O}C for 60 minutes and then add with 50µl SDS, incubated at 65^{O}C for 2 hours, after 1 hour add with 50 µl NaCl 5M and inversion every 30 minutes, centrifuged at 13000 rpm 10 minutes, water phase was moved to a new tube. DNA obtained from the water phase, add with cold isopropanol as much as 50µl, and stored overnight at 20°C. Sample was centrifuged at 13000 rpm 5 minutes, supernatan discarded. DNA pellet was cleaned with 50 µl ethanol 70%, centrifuged 13000 rpm 10 minutes. Resuspension pellet with buffer TE 50 µl.

Electrophoresis

DNA sample of 5 µl mixed with 1 µL DNA loading, resuspension and put in the well of gel agarosa 1% and connected the electrode to the power supply with 100 volts voltage for 30 minutes, then electrophoresis. The result of electrophoresis was seen on UV

transluminator and observed the ribbons of DNA visualisasion.

Fragmen Amplification of 16S rRNA and NRPS DNA genes

DNA fragments of 16S rRNA genes was amplified with PCR methods use F27 and R1492 primer, while NRPS use A3F and A7R primer. PCR for 16S rRNA genes was performed with the following program: initial denaturation at 96°C for 3 minutes, then 94°C for 1 minute, the process of anneling at 53°C for 1 minute, the extension at 72°C for 5 minutes, and the final extension at 72 ° C for 5 minutes. The program is carried out as many as 30 cycles, then stop the process by setting the temperature at 4°C (Sulistyani, 2013). Composition of materials for PCR of 16S rRNA genes are F27 primary (0,5µl), R1492 primary (0,5µl), DNA templete (10µl), nuclease free water (14µl), and green PCR (25µl).

PCR for NRPS genes was perfomed with the following program: initial denaturation at 96°C for 5 minutes, then 95°C for 30 seconds, the process of anneling at 58°C for 2 minutes, the extension at 72°C for 4 minutes, and the final extension at 72°C for 5 minutes. The program is carried out as many as 35 cycles, then stop the process by setting the temperature at 4°C (Sacido *et al*, 2005). Composition of materials for PCR of NRPS genes are KIF primary (2µl), M6R primary (2µl), DNA templete (10µl), nuclease free water (11µl), and green PCR (25µl). All of the ingredients filled an eppendrof, mixed and add mineral oil 20 µl so that evaporation does not occur. After PCR was done, mineral oil and DNA was separated then entered a new eppendorf and stored at 20°C. PCR products was visualized using electrophoresis gel agarosa 1% by putting PCR product into the well as much as 5 µl.

Analysis RFLP of NRPS genes

PCR product is cutting by restriction BsuRI (*Hae*III) enzym, then electroforesis with agarose gel 2%. Composition of materials for RFLP of NRPS genes are PCR NRPS product (7.5µl), aqua (5µl), enzim BsuRI (1u/µl) (2µl), mixed the composition, incubated overnight at 37^{O}C. Enzyme BsuRI (1u/µl) made

by mixed 2 µl of enzyme *Hae*III (BsuRI) with buffer R 18 µl.

RESULT AND DISCUSSION

DNA Isolation

DNA isolation is the first step from various DNA analysis technology. DNA can be found in the core or on chromosome organelles either. DNA isolation was broken the cell wall and nucleus membrane, followed by separation of DNA from other cell components. Electrophoresis have been done after pure DNA obtained to identify presence of DNA. Isolation of DNA results can be seen in Figure 1.

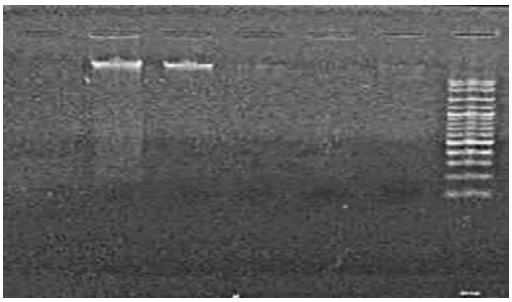

Figure 1. Results of electrophoresis of DNA isolation of actinomycetes (Wells Order: GST, KP16, T24, T37, KP, KP11, Marker)

Figure I shows that all isolates of actinomycetes can be isolated with a size more than 3000 bp. Although only KP16 and T24 isolates looks very clear fragment, while the other isolates looks very thin fragment, but can be proceed to the next steps.

Spectrophotometric UV-Vis

Spectrophotometry is an analysis method based on the measurement of UV ray absorption monokromatis by a strip of colored solution at a spesific wavelength by using a prism monokromator or diffraction grating with a fototube detector (Sudarmadji, 1996). DNA concentration can be determined using spectrophotometer UV-rays at the wavelength of 260 nm. The amount of radiation absorbed by solution (Absorban) is directly proportional to the concentration of DNA. Absorban comparison (ratio) at the wavelength of 260 and 280 nm can indicated the purity of the isolate DNA. If the ratio less than 1,8 its means that DNA isolate contamined by protein and if the ratio more than 2,0 its means that DNA isolate contamined by RNA (Stephenson, 2003). Results of Spectrophotometric antibiotic producing bacteria of isolate DNA in table I.

Table I. Measurement results OD at 260 nm and 280 nm

No	Sample	OD at 260 nm	OD at 280 nm	Purity of DNA (OD 260nm/OD 280 nm)
1.	KP16	0.0098	0.0065	1.51
2.	GST	0.0081	0.0044	1.84
3.	T24	0.0111	0.0015	7.40
4.	T37	0.0054	0.0017	3.18
5.	KP	0.0289	0.0232	1.25
6.	KP11	0.0034	0.0039	0.87

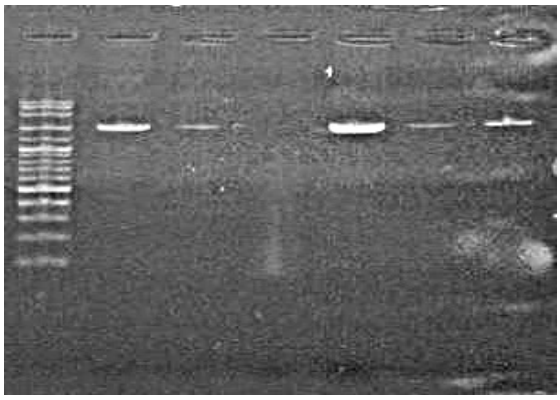

Figure 2. Results of Electrophoresis PCR DNA of 16S rRNA genes (wells order: Marker, GST, KP16, T24, T37, KP, KP11)

Purity of DNA isolation results measured by UV spectrophotometry at 260 nm and 280 nm. Based on the results KP16, KP, and KP11 have the results with a ratio less than 1,8 its means the possibility contamination by protein, and a ratio more than 2,0 possibility contamination by RNA, but tried to continued on PCR DNA 16S rRNA gene.

PCR DNA 16S rRNA GENES

PCR DNA 16S rRNA genes was done for ensure that DNA from all actinomycetes isolates can amplificated. 16S rRNA genes used as indicators of the amplification process. Results of electrophoresis PCR DNA of 16S rRNA genes in Figure 2.

Results of the PCR as showed in Figure 2 that the DNA of actinomycetes isolates are amplicable because all of 16S rRNA

genes was detected and measuring 1200 bp, except PCR product of T24, T24 fragment looks relegated, however can be continued to the process PCR of NRPS genes.

PCR DNA NRPS GENES

NRPS genes was involved in production of secondary metabolites that are important in the pharmaceutical industry. NRPS genes detection has been performed to explore the biosynthetic potential across all major actinomycetes liniages (Sacido *et al.*, 2005). The primary use for amplification of NRPS genes are A3F (forward primer) and A7R (reverse primer). This primer will stick in the adenylation domain on NRPS genes and produce PCR product that are have a measurement of 700-800 bp (Sacido *et al.*, 2005). PCR results are in Figure 3.

Based on the results of electrophoresis, all of PCR product of NRPS genes have a size of 700 bp.

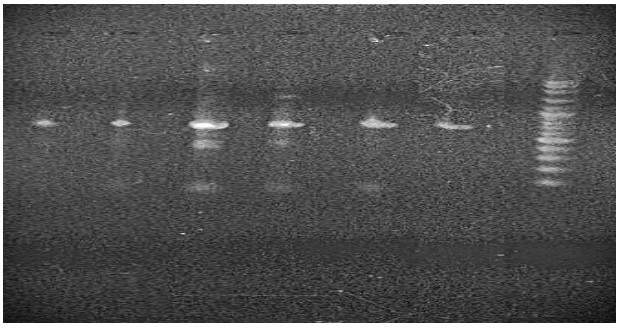

Figure 3. Results of Electrophoresis of PCR DNA of NRPS genes (wells order : GST, KP16, T24, T37, KP, KP11, Marker)

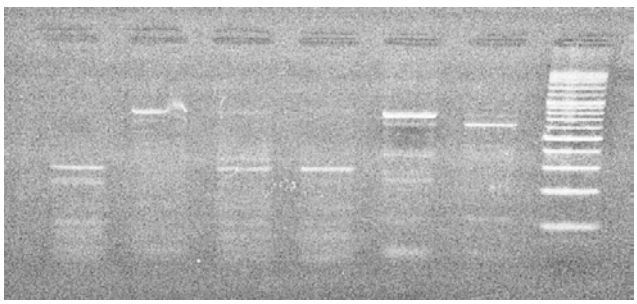

Figure 4. Results of Electrophoresis of RFLP NRPS genes (wells order: GST, KP16, T24, T37, KP, KP11, RFLP Marker)

RFLP of NRPS genes

RFLP method was used to know the cutting results of DNA fragments profile of NRPS genes of actinomycetes isolates by *Hae*III enzyme. *Hae*III enzyme cutting the DNA of NRPS genes amplification results in areas which rich in GC base pairs. This enzyme has the identification of four bases are cut in areas that have a base sequence of GGCC (Dharmaraj, 2010). Therefore, the results of cutting *Hae*III enzyme will be showed the different profiles in different actinomycetes isolates are shown in figure 4 and table II.

Diversity profiles can be observed from size of fragments by enzim *Hae*III cutting results, then th size of fragments input to UPGMA multivariate analysis with MVSP 2.0 application to array the dondrogram. The results in figure 5.

Based on the image can be grouped that T37 and GST have 64

Table II. Restriction Fragments Size Results in each PCR of NRPS genes Product

Isolat Code	Size (bp)
GST	288, 209, 102
KP16	933, 724
T24	933, 288, 102
T37	288
KP	933, 724, 389, 363, 209, 102
KP11	933, 724, 102

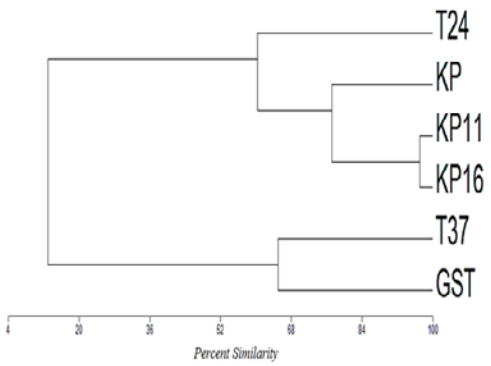

Figure 5. UPGMA Analysis Multivariate Results of the Cutting NRPS Genes By Enzyme *Hae*III

% similarity, KP11 and KP16 have 96 % similarity, KP has 76 % similarity with KP11 and KP16, while T24 has 60 % similarity with KP and 12 % similarity with T37 and GST.

CONCLUSION

Based on RFLP analysis of the NRPS genes, among of 6 actinomycetes isolates can be classified into 4 groups are T37 and GST isolates as group 1, KP11 and KP16 isolates as group 2, KP isolate as group 3, and T24 isolate as group 4.

REFERENCES

Ansari, M.Z; Yadav, G; Gokhale, R.S; and Mohanti, D., 2004, NRPS-PKS: a knowledge-besed resource for analysis of NRPS/PKS megasynthases, *Nucleic Acids Research*, Vol. 32: W405-W413.

Dharmaraj, 2010, Marine Streptomyces as a novel source of bioactive substance, *World J Microbiol Biotechnol*, 26:2123-2139.

Faizal *et al*, 2008, Polymorphism Analysis of Polyketide Synthase Gene from Actinomycetes Genome DNA of Taman Nasional Gunung Halimun Soil by Using Metagenome Method, *Journal of Biotechnology Research in Tropical Region*, Vol 1, Jakarta.

Farida,, Widada, Meiyanto, 2007, Combination Methods for Screening Marine Actinomycetes Producing Potential Compounds as Anticancer, *Indonesian Journal of Biotechnology*, Vol. 12. No. 2, pp. 988-997.

Sacido, A., Genilloud, O., 2004, New PCR Primers for the Screening of NRPS and PKS-I Systems in Actinomycetes: Detection and Distribution of These Biosynthetic Gene Sequences in Major Taxonomic Groups, *Microbial Ecology*, Spain.

Sharafi *et al*, 2012, Development and Validation of a Simple, Rapid and Inexpensive PCR RFLP Method for Genotyping of Common IL28B Polymorphisms: A Useful Pharmacogenetic Tool for Prediction of Hepatitis C Treatment Response, *Hepatitis Monthly*, 12(3):190-195

Sudarmadji, S., Suhardi., 1996, *Analisis Bahan Makanan dan Pertanian*, Liberty, Yogyakarta.

Sulistyani, N., 2013, Keragaman Isolat Actinomycetes Berdasarkan Analisis RFLP Terhadap Gen NRPS, *Jurnal Ilmiah Kefarmasian*, Vol. 3, No. 1, 2013:81-94.

Stephenson, F.H., 2003, *Calculation in Molecular Biology and Biotechnology, A Guide to Mathematics in The Laboratory*, Academic Press, California, USA.