PCR Optimization for Polyketide Synthase (PKS) and Non-Ribosomal Peptide Synthetase (NRPS) Gene Detection in Actinomycetes

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Abstract. Actinomycetes are known as a group of antimicrobial-producing bacteria. This is supported by the presence of potential genes in actinomycetes bacteria. These genes include Polyketide Synthase (PKS) and Non-Ribosomal Peptide Synthetase (NRPS). Detection of these genes using PCR requires the optimum annealing temperature so that the detection process runs accurately. The purpose of this study was to determine the appropriate annealing temperature in the detection of PKS I, PKS II and NRPS genes in actinomycetes bacterial isolates. The study was carried out experimentally with varying annealing temperatures of 52ºC and 55ºC. The results showed that all three genes were detected at 52ºC, while at 55ºC the PKS I gene bands were faint, and no PKS II and NRPS gene bands were found. Based on the results obtained, a temperature of 52ºC is a suitable temperature for the detection of PKS I, II and NRPS genes.

1 Introduction

Actinomycetes bacteria found in almost ecosystem, soil[1, 2], mangrove[3–5], and marine[6−8] environment. Exploration of actinomycetes bacteria were done due to found its potential as antimicrobial producer[9–12]. Isolation actinomycetes bacteria based on bioactive compound often similar to previously study[6]. Molecular approach to find potential actinomycetes bacteria more preferable recently[13–15].

Only few responsible gene for antimicrobial compound production were found in actinomycetes genome. Those gene were Non-Ribosomal Peptide Synthetase (NRPS) and Polyketide Synthase (PKS)[12, 16]. NRPS and PKS were genes that not only responsible for antimicrobial bioactive but also for antitumor and anticancer[17, 18]. Detecting bacterial gene based for its potential were chosen as a new promising methods for screening[19]. Distribution of NRPS and PKSs gene across the three-domain life showed not all domain posses both of gene. Domain whose posses both of gene came from Domain Bacteria with Actinobacteria as one of potential bacteria [20].

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To facilitate screening of Actinomycete bacterial inhibition using a molecular approach, PKS and NRPS genes were detected from actinomycete isolates obtained from nature using PCR and appropriate primers. The use of PCR for the amplification of the desired product is limited by the optimum conditions that must be met in the attachment of the primer to the correct DNA template. Therefore, optimizing the annealing temperature in gene detection is essential. This is because the wrong temperature will display unwanted DNA bands or even no appearance of DNA bands.

2 Experimental Details

2.1 Materials.

This study used following materials. Actinomycetes which isolated from soil of temporary dump land and estuary, microtube, micropipette, yellow tip, white tip, Thermal cycler Select Cycler II, set electrophoresis Mupid Exu Submarine (Horisontal) and UV Transilluminator Gel Doc, incubator, centrifuge, vortex, ose, bunsen burner. Reagents used in this study were DNA genome, 12,5 µL 2X PCR Master Mix (i-Taq) [buffer reaction, deoxynucleotide triphosphate (dNTP), Taq DNA polymerase, dan MgCl2], 0,5 µL Nuclease Free Water (Promega), primer set (Macrogen), Buffer TAE 10X (Promega), RedSafe Intron, agarose (Promega).

Primers used in this study were primer according to Lee [21]. Primers used in this study were A3F (5'-GCSTACSYSATSTACACSTCSGG-3') and A7R (5'-SASGTCVCCSGTSCGGTAS-3) for NRPS primers. K1F (5'-TSAAGTCSAACATCGGBCA-3') and M6R (5'-CGCAGGTTSCSGTACCAGTA-3) for PKS I primers. KSα (5'-TSGCSTGCTTCGAYGCSATC-3') and KSβ (5'-TGGAANCGCCGAABCCGCT-3') for PKS II primers.

2.2 Methods for DNA isolation.

Actinomycetes pure colony culture was transferred into a microtube containing 1 mL of Luria Bertani Broth medium and incubated at 37 °C for 24 hours. After incubation, the cultures were centrifuged at 10,000 rpm for 10 minutes. The resulting pellet was then added with 400 µl of 1X TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0), 50 µL of 1% lysozyme. The suspension was homogenized by vortexing for 30 seconds and incubated for 1.5 hours at 37 °C. 50 µL of 0.5M EDTA was added to the suspension, 50 µl of 1% SDS (w/v) and 10 µL of 0.1% (w/v) of proteinase K. The suspension was homogenized by vortexing for 30 seconds and incubated for 3 hours at 55 °C. Then, half of the total volume of 7.5 M NH_4COOH was added to the suspension and homogenized by inverting the microtube 10 times and followed by centrifugated at 12,000 rpm for 15 minutes. The supernatant formed was transferred into a new microtube and added with isopropanol (1:1 v/v). The suspension was homogenized by inverting the microtube 10 times. The suspension was stored at 20 °C for 30 minutes to precipitate the DNA. Next, the suspension was centrifuged at 12,000 rpm for 10 minutes. The supernatant formed was removed into the lysol solution. Then, the pellets formed were washed 2 times with 600 µL of cold 70% ethanol. Then, the suspension was centrifuged at 8000 rpm for 5 minutes at room temperature. The supernatant formed was then removed into the lysol solution. The resulting pellets were air-dried for 10 minutes and add 50 µL of TE buffer. After that it was stored in the freezer at -20 °C.

2.3 Methods for PCR Amplification.

The PCR reaction mixture consisted of 5 µL genomic DNA as a template, 12.5 µL 2X PCR Master Mix (i-Taq) [reaction buffer, deoxynucleotide triphosphate (dNTP), Taq DNA polymerase, and MgCl2], 0.5 µL Nuclease Free Water , and 1 µL. PCR was performed with a Select Cycler II Thermal cycler which was started at the pre-denaturation stage for 5 minutes at 94 °C; denaturation stage of 30 cycles for 40 seconds at 94°C; annealing stage for 40 seconds at 55 °C or 52 °C; extension stage for 40 seconds at 72 °C; and final extension for 5 minutes at 72 °C. The size of the PCR products are 1200−1400 bp (PKS I), 700−800 bp (NRPS), and 500−1000bp (PKS II).

2.4 Methods for Detection of NRPS and PKS gene.

PCR amplification products were analysed using electrophoresis on 2% (w/v) agarose gel dissolved in TAE 1X buffer and stained with Redsafe (2 µl for every 20 µl amplification results). Take 10 µL of DNA solution and put it in the agarose gel well. Electrophoresis was carried out with an electric voltage of 100 volts for 30 minutes. Agarose gel results were visualized using a UV transilluminator. Pictures are taken with a digital camera

3 Results and Discussion

This study already has actinomycete isolate that has been isolated previously from 2 different soil. Temporary landfill soil (code B) and estuaries soil (code A). Gene were detected in actinomycete but has different product with target gene. PKS I were found in isolate B3 and B4 (Figure 1), meanwhile PKS II found in isolate A3, A4, A6 (Figure 2). NRPS gene were detected in A3, A4, A6, B2, B3, B6 (Figure 3).

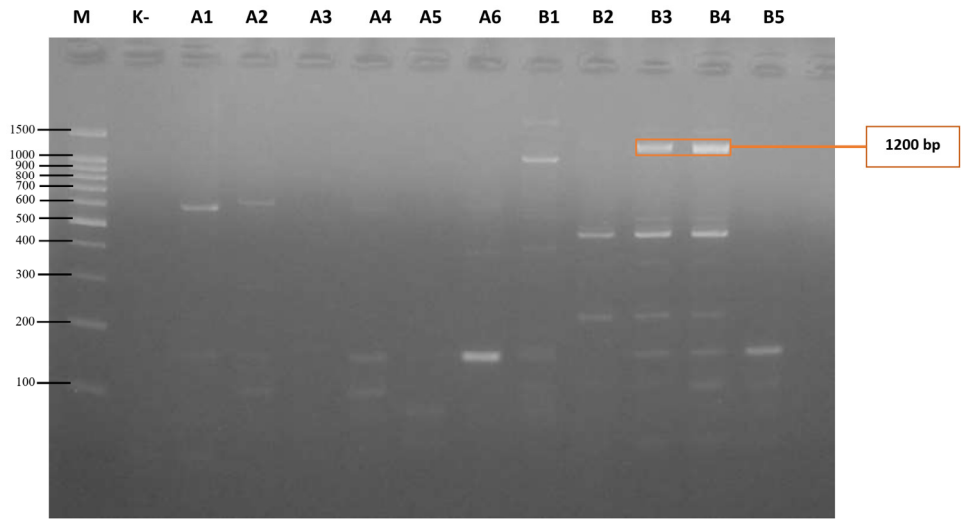

Fig. 1. Detection of PKS I gene in actinomycetes isolate at annealing temperature of 52ºC. M : marker, DNA ladder for 100bp.; K- : negative control; A1, A2, A3, A4, A5, A6, B1, B2, B3, B4, B5 : actinomycetes isolate code.

Detection of potential genes using PCR is needed to make it easier to determine the potential of bacteria to form antimicrobial metabolites. Budale et al, (2018) used an annealing temperature of 57 ºC for PKS I and II and 62 ºC for NRPS. 18.6% isolates were detected for

PKS I, 30.7% isolates were detected for PKS II. This study shows that more bands are obtained when using a temperature of 52C. 23% of isolates showed PKS I gene, 100% of isolates showed PKS II gene, and 71% of isolates showed NRPS gene.

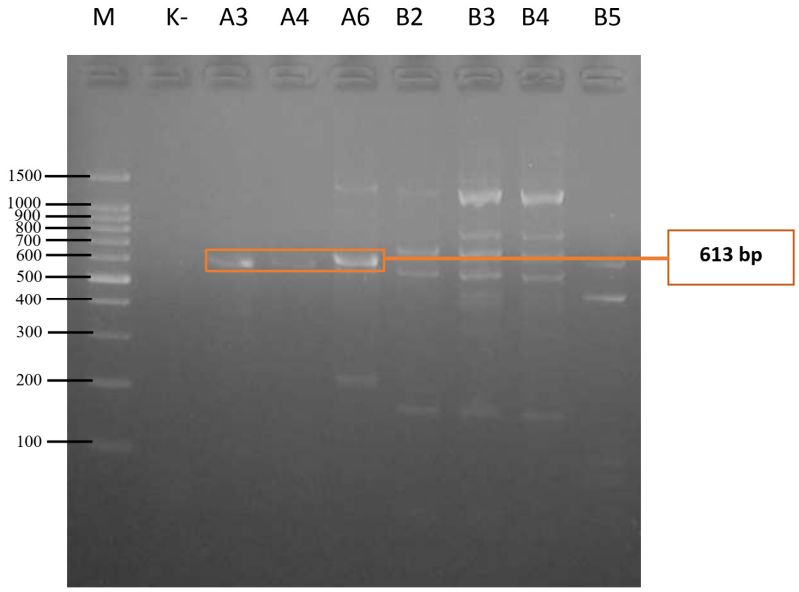

Fig. 2. Detection of PKS II gene in actinomycetes isolate at annealing temperature of 52 ºC. M : marker, DNA ladder for 100bp.; K- : negative control; A3, A4, A6, B2, B3, B4, B5 : actinomycetes isolate code.

If more than 1 band appear in one lane, can be caused by variation in primer target in result for DNA variation in bacterial species (Wood et al., 2007). Limitation of this because of model in gene bank were *Streptomyces*, while in nature not only *Streptomyces* were occupied at destined niche. Same result shown in other research [22]. Other author propose different primer from insilico study for actinobacteria identification [23].

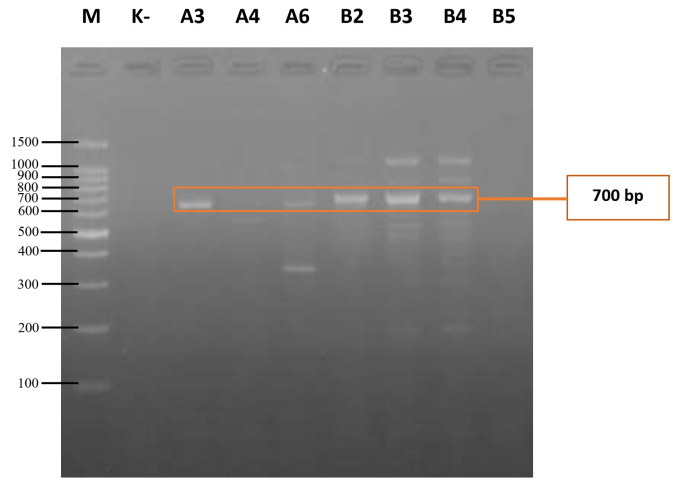

Fig. 3. Detection of NRPS gene in actinomycetes isolate at annealing temperature of 52 ºC. M : marker, DNA ladder for 100bp.; K- : negative control; A3, A4, A6, B2, B3, B4, B5 : actinomycetes isolate code.

4 SUMMARY

In summary, not all of actinomycetes isolate were exhibit NRPS, PKS I, PKS II as potential gene for producing antimicrobial metabolite. Based on the results obtained, a temperature of 52^{o}C is a suitable temperature for the detection of PKS I, II and NRPS genes. For further study, potential gene found in isolate can be used as a based to test isolate bioactivity.

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