Identification of a DNA Polymerase I Gene Fragment from a Local Isolate (PLS 80) from an Underwater Hot Spring

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Abstract

DNA polymerase is a thermostable enzyme widely used in Polymerase Chain Reaction (PCR) processes. The goal of this research was to amplify of 0.9 kb DNA polymerase I gene fragments of thermo-halophilic bacteria isolated from an underwater hot spring area, namely Pria Laot Sabang (PLS) 80. Chromosomal DNA was amplified using 1 set of primers, FP2 and RP1. The sequences of DNA polymerase I gene fragments from the PLS 80 isolate had a length of 921 bp and were identified using a direct sequencing with the NCBI BLAST tool.

The construction of a phylogenetic tree of the 0.9 kb DNA polymerase I fragment, coupled with homological analysis, showed that the PLS 80 isolate had a homology closest (99% similar) to that of Geobacillus thermoleovorans strain KCTC 3570 from the NCBI GenBank database. The results suggest that amplification of the 0.9 kb DNA polymerase gene fragment from PLS 80 isolate was 5-3'polymerase domain.

Keywords: Thermo-halophilic bacteria, DNA polymerase I gene, an underwater hot spring

Introduction

DNA polymerase is an enzyme that plays an important role in the process of DNA replication which occurs in all living cell organisms[1,2]. DNA polymerase is known to function to synthesize new DNA in 5'-3' direction. Its functions also include DNA repair, genetic recombination, reverse transcription, and production of antibodies[1].

DNA polymerase is classified into the family of Polymerase I (Pol I) or Polymerase A (Pol A), based on whether it is closely related to of *Escherichia coli* (Pol I) or human (Pol A)[2]. The enzyme is extensively used in molecular biology research, especially in Polymerase Chain Reaction techniques and dideoxy DNA sequencing processes[2-4]. Highly beneficial thermostable polymerase DNA is used in the above two methods due to its resistance to high temperatures. The thermostable enzyme can be produced by thermophilic microorganisms.

The abundance of geothermal areas in Indonesia shows a potential for isolated thermophilic microorganisms from hot springs to produce new variants of DNA polymerase[1,2]. Some research studies in Indonesia reported the potential of isolated thermophilic bacteria from Tangkuban Perahu (Domas hot spring)[1], a hot spring located at South of Bandung in West Java[2] to produce thermostable DNA polymerase.

In this study, we examined an amplified 0.9 kb DNA polymerase I gene fragment of thermo-halophilic Pria Laot Sabang 80 (PLS 80) isolated from an underwater hot spring area.

Material and Methods

Bacterial Strain: A local Pria Laot Sabang 80 (PLS 80) thermo-halophilic bacteria was isolated from an underwater hot spring area in Pria Laot Sabang, Northern Weh Island, Aceh, Indonesia. The hot spring has a temperature above 100°C, a neutral pH (7) and a salt concentration of 3.5 M.

Cultivation of Thermo-halophilic Bacteria: The bacteria isolated was cultivated in broth media ½T modification (0.4% bacto peptone, 0.2% yeast extract, 1% NaCl, 0.25% glucose, 3% bacto agar)[5]. The media was diluted with sea water. The pure culture was incubated at 70°C for 24 hours.

Isolation of a Thermo-halophilic Bacteria: The thermo-halophilic bacteria isolate was grown in 50 mL of a medium with a composition of 0.4% bacto peptone, 0.2% yeast extract, and 0.1% NaCl, 0.25% glucose. The bacteria was isolated by centrifugation of the culture. The supernatant was isolated by centrifugation of the culture for 10 minutes at 11000 *g*. The pellet cell was collected and washed twice with deionized water .

Isolation of Chromosomal DNA: The cells pellet of thermo-halophilic bacteria were suspended in 200 µL of 10 mM Tris-HCl buffer pH 8. The suspension was incubated at 37ºC for 1 hour. The cells were lysed by adding lysis buffer containing 2% SDS, 0.8 mg /mL proteinase K and 200 mM EDTA pH 8.0. The solution was incubated at 50°C for 30 min. 150 µL of a mixture of ice-cold potassium acetate and glacial acetic acid was added to the solution. The denatured proteins were precipitated by centrifugation at 6000 g for 10 minutes.

The supernatants were added twice with an equal volume of chloroform isolamilalcohol (24:1, v/v). The aqueous phase was collected by centrifugation and precipitation with 600 uL isopropanol for 5 minutes at room temperature. The

nucleic acid was precipitated by centrifugation at 16000 *g* for 20 min at room temperature. The pellet was washed twice with cold 70% ethanol and re-suspended in sterile deionized water.

Amplification and Sequencing of DNA Polymerase I Gene Fragment: A 0.9 kb gene fragment of DNA polymerase I from thermo-halophilic bacteria was amplified with PCR methods using 1 pair of primers (FP2-RP1). All PCR products were sequenced with an ABI PrismR 3100 Genetic Analyzer by a Singapore sequencing service.

Phylogenetic Analysis of DNA Polymerase I Gene Fragment: The gene fragment of DNA polymerase I was compared to fragments of DNA Polymerase sequence from the NCBI (*National Center for Biotechnology Information*) GenBank database through www.ncbi.nml.nih.gov website. The phylogenetic tree of the gene fragment of DNA polymerase was analyzed using Mega 6 software.

Results and Discussion

Amplification of Gene Fragment of DNA Polymerase: The amplification process of the gene fragment of DNA polymerase I from PLS 80 isolate was carried out using 1 set of primers (FP2-RP1) (Table 1). The FP2 primer was a specific forward primer used to conserve region (1651-1673) in the overall genetic sequence of DNA Polymerase I. The RP1 primer was a reverse primer used to conserve region (2551-2571). The result of amplification using FP2-RP1 primers successfully amplified the DNA fragment to approximately 0.9 kb in length (Figure 1).

The result of amplification of the DNA polymerase gene fragment from thermo-halophilic bacteria isolated from Pria Laot Sabang 80 show one band of DNA. The gene fragment sequencing results were analyzed using BLAST direct sequencing.

Phylogenetic Analysis: The online NCBI BLAST search tool was used to compare the homology of the DNA polymerase gene fragment with sequences from the NCBI GenBank. Analysis showed that the DNA polymerase I gene fragment from thermo-halophilic bacteria has a close homology with 35 DNA polymerase genes from microorganisms in the NCBI GenBank. DNA polymerase I gene fragment from the PLS 80 isolate showed that the isolate has a close homology to DNA polymerase fragments from five bacteria i.e. (1). *Geobacillus thermoleovorans;* (2) *Bacillus caldolyticus*; (3) *Geobacillus stearothermophilus;* (4) *Geobacillus sp* and (5) *Geobacillus kaustophilus* (Table 2).

Based on phylogenetic tree analysis, the gene fragment of DNA polymerase I from thermo-halophilic bacteria isolated from Pria Laot Sabang 80 had a homology that was most closely related to *Geobacillus thermoleovorans strain KCTC 3570*. In a phylogenetic tree, the DNA polymerase fragment showed clustering in the same branch (Figure 2).

Table 1
Primer profile of FP2 and RP1[3]

Primer	Specific Region	Sequence of Primer (5'-3')
FP2	1651-1673	AACATTAACTCGCCGAAACAGCT
RP1	2551-2571	AATCAGCTCGTCATGCACCTG

Table 2
Best 10 homologues with DNA Polymerase gene fragment from PLS 80 Isolate

Microorganisms	Accession No	% similarity
Geobacillus thermoleovorans CCB_US3_UF5, complete genome	CP003125.1	99%
Geobacillus thermoleovorans strain KCTC 3570, complete genome	CP014335.1	99%
Bacillus caldolyticus strain XM DNA polymerase gene, complete cds	EF488810.1	99%
Bacillus caldolyticus strain EA.1 DNA polymerase I (polA) gene, complete cds	AY247636.1	99%
Bacillus sp. G(2006) DNA polymerase I gene, complete cds	EF198253.1	99%
Geobacillus stearothermophilus 10, complete genome	CP008934.1	99%
Geobacillus sp. 777 DNA-directed DNA polymerase (polA) gene, partial cds	KP993175.1	99%
Geobacillus sp. GHH01, complete genome	CP004008.1	99%
Geobacillus kaustophilus HTA426 DNA, complete genome	BA000043.1	99%
Geobacillus sp. JS12, complete genome	CP014749.1	99%

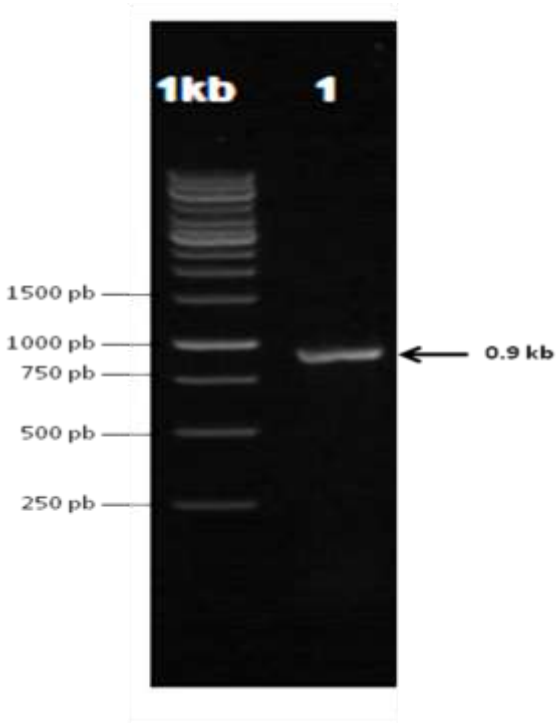

Figure 1: Electroforegram of 0.9 kb gene fragment of DNA polymerase from PLS 80 isolate. 1kb. DNA Marker; 1. 0.9 kb gene fragment of DNA polymerase

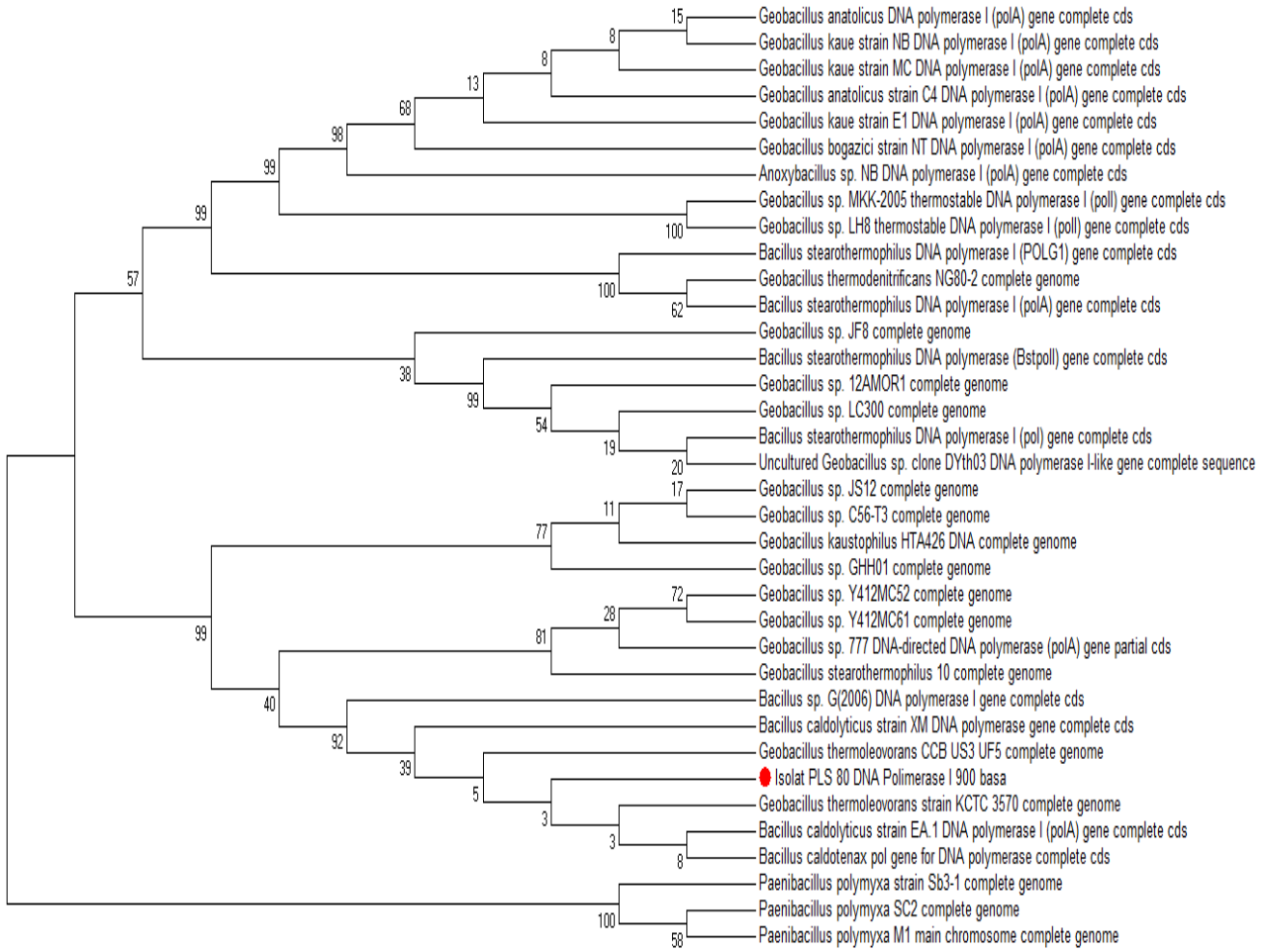

Figure 2: Phylogenetic tree relationships of gene fragment of DNA polymerase of the PLS 80 isolate in relation to 37 sequences using the neighbor-joining method in MEGA 6 with 1000 bootstrap replicates.

Further analysis obtained from protein structure modeling of the DNA polymerase fragment showed that the PLS 80 isolate has a homology closest to DNA Polymerase I from *Geobacillus stearothermophilus*. A sequence alignment of the protein structure of the DNA polymerase I fragment from PLS 80 isolate was performed with a *Geobacillus stearothermophilus* template. The protein structure showed that the gene fragment contained the 5'-3' polymerase domain. This domain plays a significant role in the polymerization of DNA.

Conclusion

The homology of the 0.9 kb gene fragment of DNA polymerase I from PLS 80 isolate showed that the isolate was closely related to *Geobacillus thermoleovorans*, with a sequence similarity value of 99%, based on genetic data from the NCBI GenBank. The results showed that this fragment was part of the DNA polymerase domain and a homologue with the DNA polymerase domain of *Geobacillus stearothermophilus.*

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