ISSN: 978-602-71169-7-9

Novel Archaeal DNA Polymerase B from Domas Hot Spring West Java

Suharti[1,2], Rukman Hertadi[1], Fida Madayanti Warganegara[1], Santi Nurbaiti[1] and Akhmaloka[1*]

[1]Biochemistry Research Group Faculty of Mathematics and Natural Sciences

Institiut Teknologi Bandung, Jl Ganesha 10 Bandung 40135 Indonesia

[2]Faculty of Pharmacy Universitas 17 Agustus 1945 (UTA'45) Jakarta, Jl Sunter Permai Raya Sunter Agung Jakarta Indonesia

*corresponding author: loka@chem.itb.ac.id

Abstract. Nine novel archaeal DNA polymerase genes from Domas Hot Spring, West Java have been cloned directly through the natural sample. The characterization of the genes showed that the genes are high homology to the DNA polymerase B of Crenarhaea phyla. Phylogenetic analysis of the amino acid sequences showed that the enzymes are grouped in a new branch from the other Crenarchaea's DNA Polymerase B. 3D structure analysis of the enzymes show that the structures are closed to the structure of DNA Polymerase B1 from *Sulfolobus solfataricus*. The nine structures of the enzymes could be grouped into four different structures.

Keywords: DNA Polymerase B, Crenarchaea, Domas Hot Spring

Introduction

Studies on DNA Polymerase as one of the replication enzymes has went through rapid progress in the third last decades. Modifications of this enzyme reveal products that are beneficial for the biotechnological expansion. As a key in replication and DNA modification process, good polymerization and exonuclease activity or processivity is really necessary. Therefore, the searching for new variant of DNA Polymerases from the extreme environment are still being done. Various efforts have been made to obtain a new variant of good quality DNA Polymerase, such as by isolating the DNA polymerase gene from extremophiles microbes that have been cultivated. However, to cultivate extremophiles microbes in a laboratory is hard to do. Less than 1 % of microbe from the environment can be cultivated in the laboratory [1]. So, it is necessary to find other techniques or approaches to obtain new variant DNA polymerases. In this report we describe the obtaining of new variant of thermostable DNA polymerases from metagenomic sample which were taken from the extreme environment of Domas Crater West Java.

Materials and methods

The microbial samples were collected from hot spring water at Tangkuban Perahu Crater, West Java. The spring temperature is around at 92oC to 95oC and pH is around at 1 to 2 respectively. The water then gently filtered through 0.2μm Millipore filter membrane to collect the cell. Total DNA samples were isolated based on Zhou et al. [2] with slight modification.

PCR reaction used Ssofast supermix, (BioRad). A total of 20 μL PCR reaction mixture (10μL Ssofast, 2 pmol primer pair, 1 μL of community DNA as template and ddH2O) was used for the amplification. The PCR was carried out with an initial denaturation step at a temperature at 98oC for 7 minutes, each cycle of denaturation at 98oC for 30s, annealing temperature with a gradient temperature from 56°C – 46°C for 30s, extension at 72°C 3 min for 31 cycles, final extension at 72°C for 10 min and cooling at 12oC 10 minutes.

pJET12/blunt cloning kit (Fermentas) was used for cloning of PCR product. The ligation reaction was performed according to the kit manual. The ligation product then used to transform Escherichia coli Top10 (Invitrogen).

This Proceeding © Faculty of Sciences and Mathematics
Diponegoro University, 2015

DNA sequencing was performed by using the Dye Terminator (3'-dye labeled dideoxynucleotide triphosphate) which includes several stages, namely: template preparation, sequencing reaction, PCR product purification, electrophoresis and scanning fluorescence.

Homological analysis were performed by aligning the DNA sequences with NCBI data using BLASTN program. Prior to the homology analysis, sequences were exposed to ORF finder to determine the coding region. The coding region was then translated in silico based on BLASTP program. Alignment of amino acid sequences was performed by ClustalX program and visualized using Genedoc program. Phylogenetic tree was constructed based on maximum likehood method using Jones-Taylor-Thornton (JTT) model with Mega5 [3].

Three dimensional structure modeling was performed by superposition of amino acid sequence with protein structures in the Protein Data Bank (PDB).The amino acid sequence of the samples were submitted to the Swiss-Model server [4]. Visualizing of 3-dimensional models was used PyMol viewer program.

Results

Total DNA community was isolated directly without the cultivation process from Domas Hot Spring sample. Cloning of DNA polymerase genes of metagenomic sample was carried out in vivo in Escherichia coli cell, which previously have been amplified in vitro by using PCR technique from total community chromosomal DNA of the water. Some pairs of primer have been designed to amplify DNA polymerase gene. First, internal primer pair were designed to amplify approximately 0.4 kb gene fragment of DNA polymerase inside polymerase domain; while the external primers amplify 2.7 kb fragments. The internal fragment amplified 0.4 kb while the external surprisingly amplified two fragments with the size at around 3 kb and 6 kb. The amplicon of the internal primer were then cloned and sequenced. All of the sequences of the internal fragment confirm as part of DNA polymerase coding region. Both amplicons from the external primer were also cloned and sequenced. The 6 kb inserts of the clones have been in silico translated and their alignment analysis showed that nine clones (bearing 6 kb fragment) containing 2.6 kb structural gene of DNA polymerase B.

Alignment analysis of the amino acid sequence of the samples by using NCBI BLAST program reveal that the amino acid sequence of the nine samples have high homology with the amino acid sequence of DNA polymerase I Metallosphaera sedula. The next close homology with the DNA Polymerase of the phylum Crenarchaea. These amino acid sequences have varied homology from lows of 90% and the highest 92% homology to the DNA Polymerase I Metallosphaera sedula.

Alignment with about 100 amino acid sequence of GenBank showed that the amino acid sequence homology with the DNA sample has Polymerase archaeal groups with an error rate (E value) is very low (0.0 to 2x10-45). Lowest homology of the amino acid sequence of samples is the amino acid sequence of DNA Polymerase Pol2 [Vulcanisaeta moutnovskia 768-28] with access number YP_004245486.1. Data of alignment of amino acid sequence of this protein sample to [max score of 184, a total score of 184, query coverage 81%, the E value 2x10-45, Identity 28%]. This suggests that the amino acid sequence of samples is correctly aligned DNA Polymerase B from the group of archaea.

Identity between the amino acid sequence of each sample shows that the highest similarity owned by the sample no. 02 with sample no. 05 with a rate of 97.7% identity. Whereas the lowest similarity owned by the sample no. 03 with sample no. 08 with a rate of 94.1% identity. Thus it can be said that the samples are different. Samples with the lowest homology is sample no. 03 which has an average homology with other samples of about 95% followed by no. 09 samples with levels of about 96% homology. Samples with high homology with other samples are samples with no. 02 and sample no. 01 which has an average degree of homology of about 97%.

Analysis of the amino acid sequence with the construction of a phylogenetic tree based on the sequence of DNA polymerase from group

This Proceeding © Faculty of Sciences and Mathematics
Diponegoro University, 2015

ISSN: 978-602-71169-7-9

B archaea shows that the amino acid sequence of the samples included in the family DNA Polymerase B (B1) from the phylum Crenarchaea. From the phylogenetic tree is seen that each sample was separated into different branches, although still in one group. This indicates that the DNA polymerase and DNA Polymerase obtained is different. From this tree is also seen that the group of the amino acid sequence of samples to form a branch with DNA Polymerase I Metallosphaera sedula, this suggests that the DNA Pol and Pol B DNA sample is different with the DNA polymerase I from Metallosphaera sedula.

Modeling of 3D Structural from hypothetical protein samples was done by sending samples to the amino acid sequence server Swiss Model (http://www.expasy.org/Swiss-Model). Visualization of 3D models is done using PyMOL viewer (Schrödinger, Inc.). Modeling using Swiss server model is based on the highest structural homology with the template structure that found on PDB data. Nine hypothetical proteins DNA polymerase B samples have been modeled three-dimensional structure; the template used in this model is the 3D structure of the crystal structure of DNA Polymerase B1 Sulfolobus solfataricus, with PBD id 1S5JA.

Based on homology prints are given, not all of the amino acid sequence of samples can be displayed in a 3D structure, because the crystal structure of the mold is also incomplete. This is caused by the loss of amino acid residues at the time of formation of crystals [5]. In general, all the 3D structure of divided into two folds, namely the N terminal and C terminal. Folds C terminal describe right palm, with three main parts: thumbs, palms and fingers which is the domain of the polymerase. On the palms and fingers, there are contain the active polymerase. At the crease exonuclease N terminal domain and subdomains are N terminal. On the side there are exonuclease domain ligand binding Mg2+, and the N terminal subdomain contained uracil binding pocket (uracil binding pocket).

By modeling the 3D structure of the sample, there is a striking difference in the 3D structure of the nine samples. Therefore, of the nine models of the 3D structure of the sample can be classified into four groups: 1. The normal structure of the structure that fits the template, 2. The structure of the ligand binding in the absence of Mg2+; 3. The structure with the addition of β sheet in front of the polymerase domain without the ligand binding Mg2+; and 4. The structure with the addition of β sheet in front of the polymerase domain with the ligand binding Mg2+.

References

[1] Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev*, **68**: 669-685.

[2] Zhou J, Bruns MA and Tiedje JM (1996) DNA Recovery from Soils of Diverse Composition. *Appl Environ Microbiol*, **62**: 316-322.

[3] Hall BG (2013) Building Phylogenetic Trees from Molecular Data with MEGA. *Mol Biol Evol.,* **30**(5): 1229-1235.

[4] Rost B, Yachdav G and Liu J (2004) The PredictProtein server. *Nucl. Acids Res.* **32** (2): W321-W326.

[5] Savino C, Federici L, Johnson KA, Vallone B, Nastopoulos V, Rossi M, Pisani FM, Tsernoglou D (2004) Insights into DNA replication: the crystal structure of DNA polymerase B1 from the archaeon *Sulfolobus solfataricus*. *Structure,* **12**(11):2001-8.

Acknowledgement

This research was funded by: ITB excellent research - directorate general of higher education, Ministry of research, technology and higher education 2015.

This Proceeding © Faculty of Sciences and Mathematics
Diponegoro University, 2015