Regular

International Journal of Integrative Biology
A journal for biology beyond borders

IJIB
ISSN 0973-8363

Cloning, Homological Analysis, and Expression of DNA Pol I from *Geobacillus thermoleovorans*

Akhmaloka [1,*], Hendro Pramono [2], Laksmi Ambarsari [3], Evi Susanti [4], Santi Nurbaiti [1], Fida Madayanti [1]

[1] *Biochemistry Research Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Indonesia*
[2] *Faculty of Biology, University of General Soedirman, Purwokerto, Indonesia*
[3] *Department of Biochemistry, Bogor Agricultural University, Bogor, Indonesia*
[4] *Department of Chemistry, State University of Malang, Malang Indonesia*

Submitted: 4 Jan. 2008; Accepted: 26 Jan. 2008

Abstract

DNA POLI gene from *Geobacillus thermoleovorans* (*DNA POLI ITB$_1$*) was cloned and overexpressed in *Escherichia coli* using two steps PCR amplification. The gene was isolated and sequenced to consist of 2628 nucleotides that encode 876 amino acids. The deduced amino acid sequence of DNA Pol1 ITB$_1$ showed high similarity to all known DNA polymerase I, with the highest homology to DNA Pol1 from *Bacillus caldotenax*. Homological analysis revealed that the protein sequence contained all of the motifs conserved in the family of DNA Pol1 for 5'→ 3' exonuclease and polymerase activities. However, the motif for 3'→ 5' exonuclease was less conserved as known on DNA Pol1 from the genus of *Bacillus*. The gene was overexpressed in *Escherichia coli* as fusion protein with thioredoxine at the C-terminus. It was purified by heat treatment, followed by ammonium sulfate fractionation and ion exchange chromatography. The molecular weight of purified fusion protein was approximately 110 kDa, as shown by SDS-PAGE and still showed polymerase activity. The optimum pH and temperature of polymerase activity were at 7.5 and 65^{o}C.

Keywords: Thermostable, DNA Pol1, *Geobacillus thermoleovorans*, *Bacillus caldotenax*, overexpressed, fusion protein

INTRODUCTION

DNA polymerase plays a leading role in the replication and maintenance of the genome and are central to the accurate transmission of genetic information from generation to generation that is presented in all living cells. DNA polymerase was first isolated by Kornberg from *Escherichia coli* (Kornberg *et al.*, 1992). The enzyme was a single chain polypeptide with multifunction activities, polymerase 5'→ 3', exonuclease 5'→ 3' and exonuclease 3'→ 5' activities (Joyce *et al.*, 1994). Up to today more than 100 DNA polymerases have been isolated and studied.

Initially, DNA polymerases were classified as either Pol I family or Pol A family on the basis of similarity to *E.coli* Pol1 or human Pol A. A new DNA polymerases nomenclature has been suggested based on sequence similarity, DNA polymerases can be classified into A, B, C, D, X, Y and RT families (Patel *et al.*, 2001). Perhaps the most extensively studied of DNA polymerases are those in family A, the *E.coli* DNA Pol1 and *Thermus aquaticus* DNA Pol1, whose amino acid sequences and crystal structures are known (Steitz, 1999).

DNA polymerases have been used extensively in molecular biological research, especially in two of most important methods: the PCR technique and dideoxy DNA cycle sequencing. Thermostable DNA polymerases, such as *Taq*

[*] ***Corresponding author:***
Akhmaloka, Ph.D.
Biochemistry Research Division,,
Faculty of Mathematics and Natural Sciences,
Institut Teknologi Bandung Jln Ganesha 10
Bandung, 40132, Indonesia
Email: loka@chem.itb.ac.id

DNA Pol, have been the key element in the development of the polymerase chain reaction (Saiki *et al.,* 1988; Mullis *et al.,* 1986). *Taq* DNA Pol (from *T. aquaticus*) was first characterized thermostable enzyme (Chien *et al.,* 1976). Then after many of DNA Pol from other *Thermus* strains were studied. *Taq* and other DNA polymerases from the *Thermus* genus lack the 3'→ 5' exonuclease proofreading activity of the *E. coli* homologue (Chien *et al.,* 1976). On the other hand, a highly thermostable Pol1 from the hyperthermophiles contains all three function of the *E.coli* Pol1 (Perler *et al.,* 1996). The enzyme has 3'→ 5' exonuclease activity dependent proofreading activity. The activity is required for error correction during the polymerization. Several thermostable DNA polymerases with proofreading activity, such as *Pfu*, *Vent*, *Deep Vent* and *Pwo* have also been studied and introduce for high-fidelity PCR amplification (Cariello *et al.,* 1991; Lumberg *et al.,* 1991; Frey *et al.*, 1995).

A few moderatetly thermostable DNA polymerases have been isolated and purified from thermophilic *Bacillus* species (Akhmaloka *et al.*, 2006; Perler *et al.,* 1996). *Bst* DNA Polymerases was isolated from *B. stearothermophilus* (Stenesh *et al.*, 1972; Kaboev *et al.,* 1981; Sellmann *et al.,* 1992). *Bca* DNA polymerase was isolated and cloned from *B. caldotenax* (Sellmann *et al.,* 1992; Uemori *et al.*, 1993). *Bst* DNA polymerase has been used for DNA sequencing.

Most of the native enzymes are synthesized at very low levels by the thermophilic microorganisms, therefore, they are cumbersome to purify. Most of thermostable DNA polymerases were produced in a biologically active form in *E.coli* expression system (Blondal *et al.,* 2001; Kim *et al.,* 2002; Choi *et al.*, 2004; Shin *et al.*, 2005; Kim *et al.*, 2007). However, several problem persist, such us error-prone amplification and unwanted amplification at low temperatures. New and improved thermostable DNA polymerases are needed.

In this report we described cloning and characterization of a gene that encodes DNA polymerase from *Geobacillus thermoleovorans* (Indrajaya *et al.,* 2003). The thermostable DNA polymerase from the cloned gene was over-expressed in *E.coli* and characterized.

MATERIALS AND METHODS

Bacterial strains and plasmids

The strain used for cloning was *E. coli* JM109 (*recA1*, *endA1*, *gyrA96*, *thi*, *hsdr17* ($r_{k-}m_{k+}$), *relA1*, *supE44*, Δ(*lac-proAB*), [*F'*, *traD36*, *proAB*, *laclqZΔM15*]). *E.coli* GI724 (F^-, lamda$^-$, *lac*PL8, *amp*C::P_{trp}*cl*) was used for heterologous expression. The plasmid used for cloning vector was pGEM-T® (Promega), while pTrxFus™ (Invitrogen) and pTrx ™ (Invitrogen) were used for expression vector and the control.

Growth condition and DNA manipulation

G. thermoleovoran was isolated from Hot Spring, located at South of Bandung, West Java, Indonesia. The isolate was grown at 70^0C in medium containing 0.2% yeast extract (Sigma), 0.4% peptone (Sigma), 0.01% $(NH_4)_2SO_4$, 0.025% $MgSO_4.7H_2O$, 0.0125% $CaCl_2$, 0.03% KH_2PO_4 and 0.1% NaCl (Indrajaya *et al.,* 2003). *E. coli* strains were grown at 37^0C in Luria Bertani (LB) broth or plated on LB agar containing the appropriate antibiotics when required. For *E. coli* GI724 was grown at 37^0C on RMG-Amp medium (1x M9 salts, 2% casamino acid, 0.5% glucose, 1mM $MgCl_2$, 100 µg/mL ampicillin). All routine DNA isolation and manipulations were performed as described by Sambrook *et al*. (1991). Restriction enzymes and other modifying enzymes were purchased from Promega (Madison, WI, USA). Small-scale preparation of plasmid DNA from *E. coli* cells was performed using plasmid mini-prep kit (Qiagen, Hilden, Germany).

Preparation of Primers and PCR

From an amino acid sequence alignment of known bacterial DNA polymerases, highly conserved sequences were selected in order to make internal primers. The primers were designed based on the conserved sequences. The sense primer (FP1) was 5-gat ccg aac ctg caa aac att cc-3'. The antisense primer (RP1) was 5'-cag gtg cat gac gag ctg att-3'. The external primers were designed based on the DNA sequences obtained from internal region of the gene resulted from amplification using internal primers. The sense primer (FP4) was 5'-gtt ggg aga gcg ttc aag gca agc gtc-3', and the antisense primer (RP4) was 5'-gga gtc ttg gtg tgt gga tcg c-3'. A polymerase chain reaction (PCR) was

performed in total volume of 50 µl using PCR system 2400 (Aplied Biosystems, Foster City, USA). The mixture contained 0.5 unit *Taq* DNA polymerase, 5 µl of 10 X *Taq* DNA polymerase buffer, 0.2 mM each of dNTP, 100 pmol of each primer, and 0.5 µg of *G. thermoleovorans* genomic DNA. PCR was performed by 30 amplification cycles on the condition, denaturation (96^{o}C, 1 min), annealing (45-50^{o}C, 1 min) and extension (72^{o}C, 1 min). After the final cycle, the reaction mixture was kept at 72^{o}C for 5 min.

Sequence analysis

Sequence of double stranded DNA were determined using a Dye Terminator Cycle Sequencing Ready Mix (Aplied Biosystem, Foster City, USA) on PCR System 2400 and ABI PRISM 310 Genetic Analyzer (Applied Biosystem, Foster City, USA). To sequence the whole coding region of the gene, the cloned plasmid was used as a source of the template DNA. Sequence comparisons were performed using the BLAST program (Altschul *et al.*, 1990).

Computer Analysis

The computer-assisted DNA and protein sequence analysis was performed using GeneDoc version 2.6.002. The BLAST program at the NCBI server was used for sequence similarity.

Construction of the expression plasmid

For the expression of DNA polymerase, two primers (FPK and RPBZ) were designed. The 5' end primer (5'-ggt acc aat gaa aaa aaa gct tgt ttt aat c-3') contained the *Kpn*I restriction enzyme site and the 3' end primer (5'-taa gag gat cct tta tgt cgc gtc ata cc-3') contained the *BamH*I restriction enzyme site. The primers were used to amplify almost the whole coding region of the gene with additional *Kpn*I and *BamH*I restriction sites. PCR was performed for 30 amplification cycles under the following condition: denaturation (95^{o}C, 1 min), annealing (55^{o}C, 1 min) and extension (72^{o}C, 1 min). After the final cycle, the reaction mixture was kept at 72^{o}C for 5 min. The 2.7 kb amplified PCR product was identified on the 1% agarose gel. The PCR product was eluted from the gel and digested with *Kpn*I and *BamH*I. The pTrxFus™ (Invitrogen) expression vector was digested with *Kpn*I and *BamH*I in advance. The 2.7 kb PCR product and vector were ligated with T4 DNA Ligase (Promega) at 16^{o}C overnight. The recombinant plasmid was introduced into *E.coli* GI724 by the heat-shock method. The cloned 2.7 kb gene was identified by sequencing.

Expression and Purification of DNA polymerase

DNA polymerase was expressed in *E. coli* GI724. A single colony of *E. coli* GI724 was picked and inoculated to RM-A media (M9 salt 1x, cassamino acids 20% (w/v), 1.0% glycerol (w/v), 1mM $MgCl_2$ and 100 mg/mL amphicilin). The culture was incubated at 30^{o}C with gentle shaking (200 rpm) for overnight. The culture was re-inoculated into induction medium (M9 salt 1x, 0.2% (w/v) cassamino acids, 0.5% (w/v) glucose, 1mM $MgCl_2$ and 100 mg/mL amphicilin) with OD_{550} = 0.1 and incubated at 30^{o}C with gentle shaking until OD_{550} = 0.5 reached. The culture was induced with 100 µg/mL tryptophan and incubated at 37^{o}C for 3 hour. The cell were harvested by centrifugation (5000 x g at 4^{o}C for 10 min) and resuspended twice in lysis buffer (50 mM KCl, 10 mM Tris-HCl pH 7.4, 0.1% Triton X-100, 2.5 mM $MgCl_2$). The cells were sonicated five times for 30 s each time with 30 s intervals. The cell debris was removed by centrifugation (15000 x g at 4^{o}C for 30 min). The supernatant was transferred to a 15 mL conical tube and incubated in an 80^{o}C water bath with shaking for 10 min. The heat-treated supernatant was cooled on ice for 20 min, and then centrifuged (18000 x g at at 4^{o}C for 30 min) to remove the denatured *E. coli* proteins. After removal of the denatured proteins by centrifugation, the supernatant was fractionated with ammonium sulfate, using 20, 40, 60 and 80% saturations. The pellet obtained with 40-60% saturation was resuspended on buffer A (20 mM Tris-Cl pH 8.0 and 2.5 mM EDTA), dialysed for 12-16 h against 100 x volumes of same buffer and applied to DEAE-selulose column that had been equilibrated with buffer B (50 mM fosfate buffer pH 6.0, 2 mM mercaptoetanol, 10% glycerol, 4 µM PSMF and 2.5 mM EDTA). The elution of adsorbed protein was performed with linier gradient of NaCl (10-500 mM) in buffer B. The fraction showing DNA polymerase activity was pooled and dialysis against buffer A. The purified protein was then analysis and assayed.

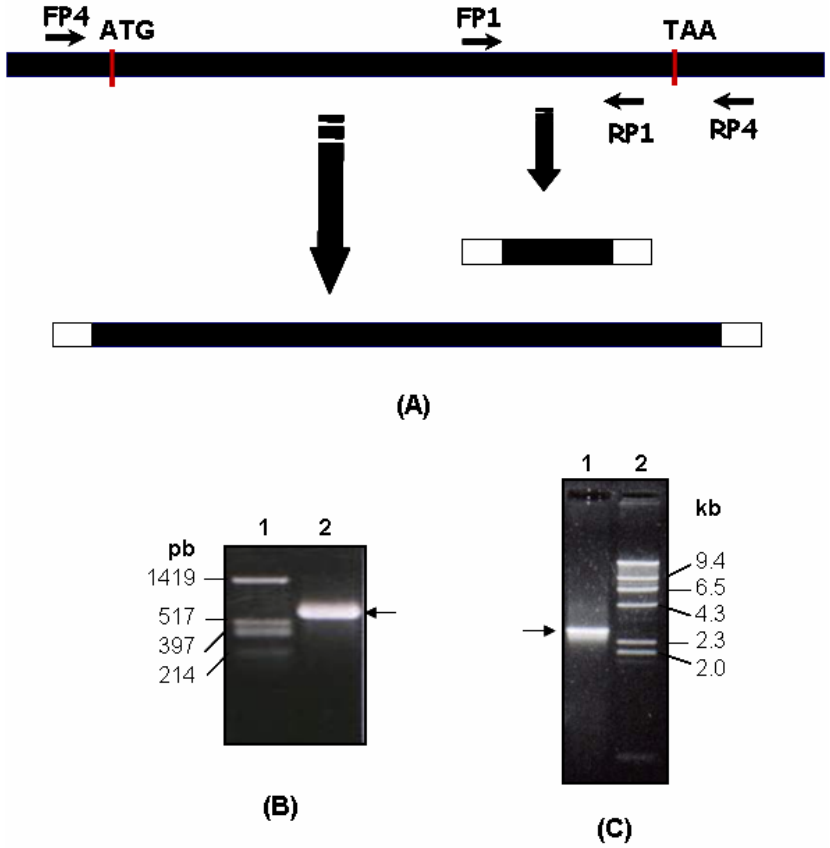

Figure 1: Cloning strategy and amplification of DNA Pol1 ITB$_1$
(A) Two step of PCR strategy to amplified the whole coding region of *DNA PolI* ITB1. FP1 and RP1 are the internal primers, while FP4 and RP4 are the external primers. (B). Electroforegram of internal amplification of DNA PolI fragment. Lane 1 is DNA marker (pUC19 cut by *Hinf* I). Lane 2 is the 600 bp fragment. (C). Electroforegram of external amplification of the whole coding region of DNA PolI ITB1. Lane 1 is the 2.7 kb fragment of DNA PolI ITB1. Lane 2 is λ DNA was cut by *Hind* III as marker.

DNA polymerase Assay

The DNA polymerase activity was measured by the modified assay method that was described previously (Kahler *et al.*, 2000). The method was based on non-radioactive assay. A 100 µL mixture contained 50 mM Tris-Cl (pH 7.3), 0.5 mM $MgCl_2$, 60 µg of BSA, 0.36 µM DIG-11-dUTP, 18 µM biotin-11-dUTP, 18 µM dTTP, 1.2 µg DNA I-activated calf thymus DNA as a template, and DNA polymerase samples. The reaction mixture was incubated 75°C for 1h. One unit of DNA polymerase was defined as the amount of enzyme required to incorporate 120 fmol of DIG-11-dUTP per 30 min at 75°C. *Taq* polymerase was used for the production of calibration curves, with 50 mM Tris-Cl (pH 7.9), 5 mM$MgCl_2$ and 50 mM KCl in the reaction mixture.

Effect of pH and temperature on DNA polymerase activity

The recombinant DNA polymerase was investigated for optimal reaction condition using DNA polymerase assay, as described previously. The activity of the enzyme was measured in different condition of pH and temperatures.

RESULTS

Cloning and Sequence Analysis of *DNA POL1 ITB1*

To steps of PCR methods have been used to clone *DNA POLI* gene from *G. thermoleovorans* (Fig. 1A). A DNA fragment of approximately 640 bp in length was amplified from *G. thermoleovorans* genomic DNA by PCR with internal primer based on conserved region of polymerase domain in bacterial DNA polymerases (Fig. 1B). The PCR product was cloned in pGEMT-easy vector and sequenced. The sequence of PCR product was homologous with those of DNA polymerases gene from other

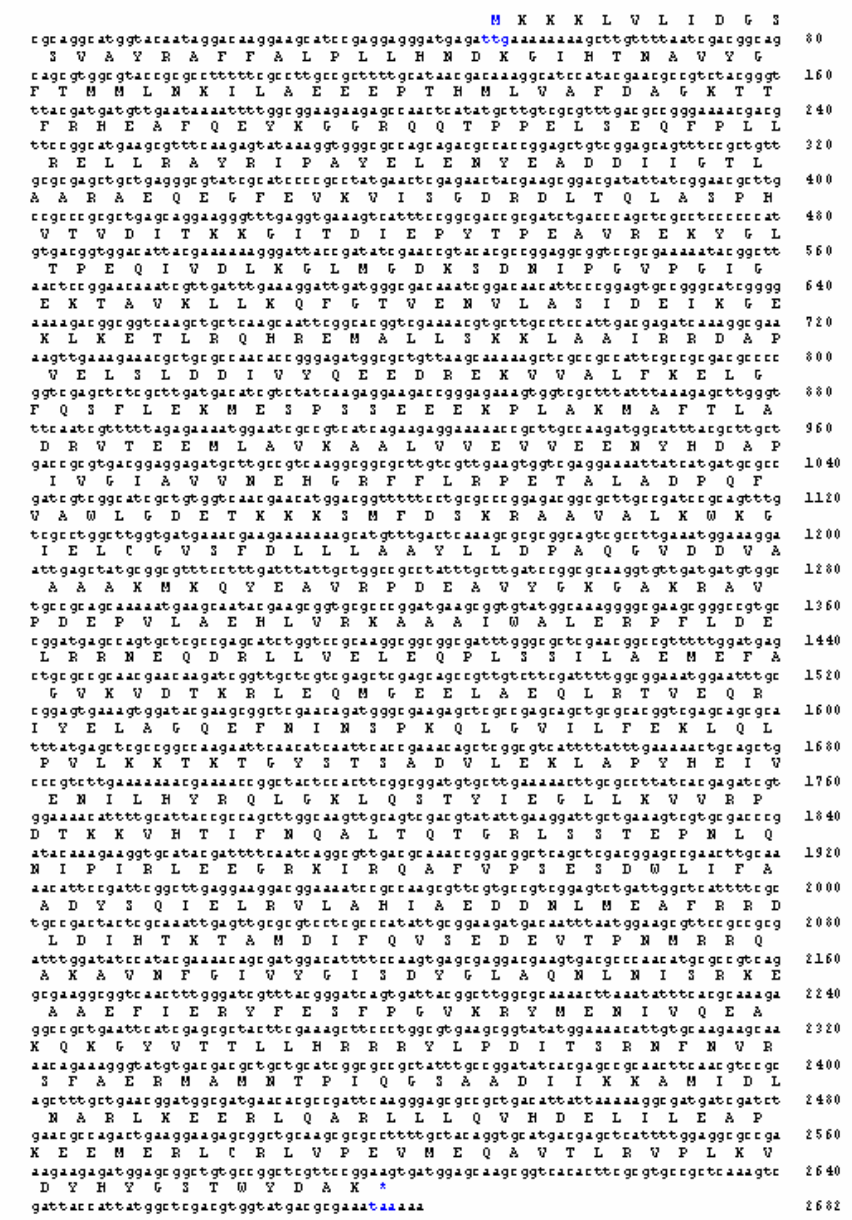

Figure 2. Nucleotide sequence of the polymerase gene and deduced amino acid sequence (876 amino acids). The TTG start and TAA termination codons are illustrated on blue.

bacteria (data not shown). Based on the sequence resulted, the external primers which designed to amplify the whole coding region of the gene were constructed. The external primer was successfully amplified DNA fragment of approximately 2.7 kb in length (Fig. 1C). The amplicon was cloned to pGEMT-easy vector and sequenced. The complete nucleotide sequence of *DNA POLI* ITB1 consists of 2,628 nucleotides and 876 deduced amino acids (Fig. 2).

Homological Analysis of Deduced Amino Acid of *DNA Pol1 ITB1*

The deduced primary structure of DNA Pol1 ITB_1 was aligned and compared with those of bacterial DNA Pol1. Amino acid sequence alignment revealed that DNA Pol1 ITB_1 contains 5'→3' exonuclease (1-296), 3'→5' exonuclease (297-468), and 5'→3' polymerase (469-876) domains. The 5'→3' exonuclease and

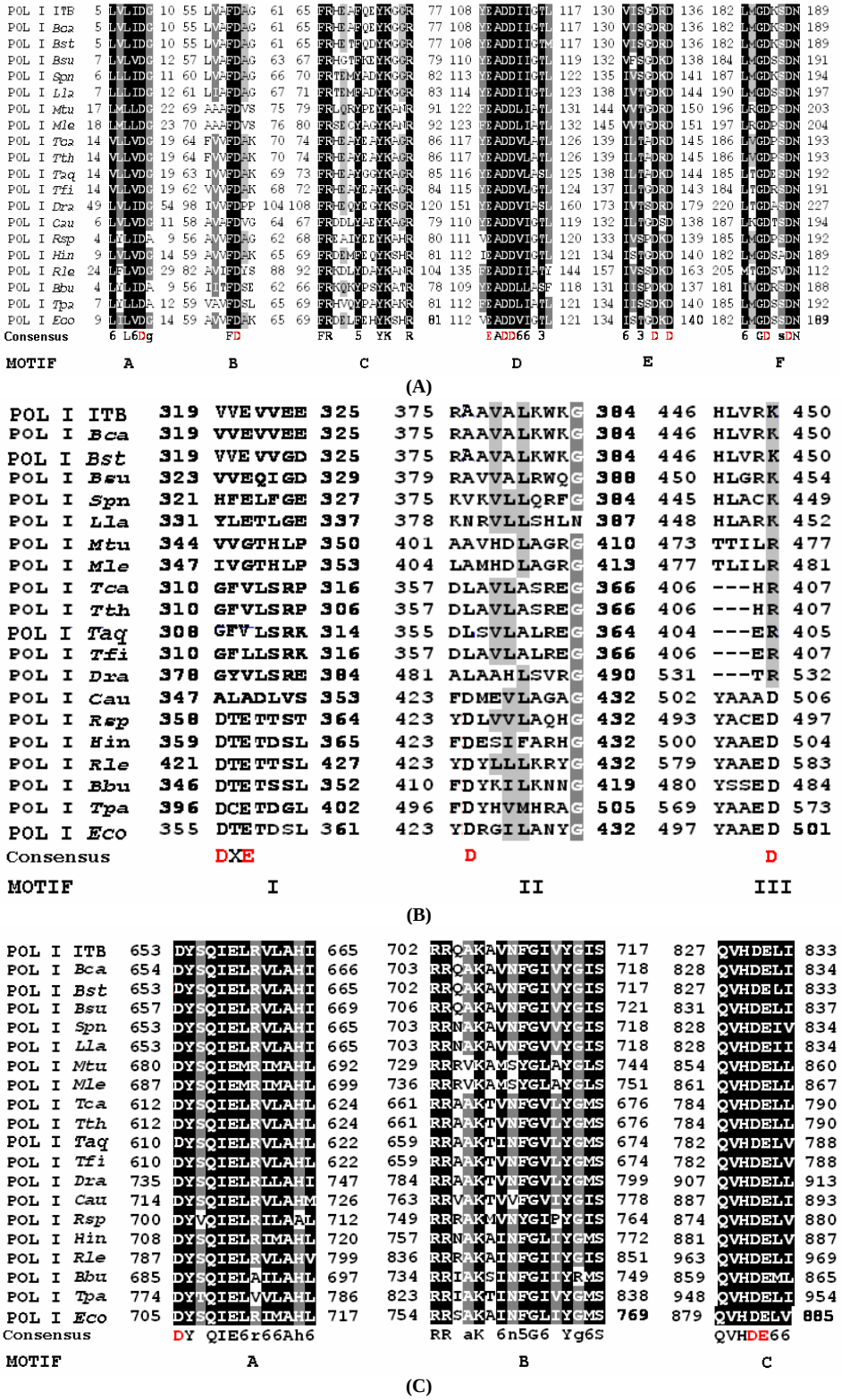

Figure 3. Homological analysis of DNA PolI ITB1 with other DNA PolI from various organisms. (A). 5'-3'exonuclease domain, there are 6 consensus motifs (A,B,C,D,E and F). (B). 3'-5' exonuclease domain, there are 3 mptifs (I, II and III). (C) 5'-3' polymerase domain, there are 3 motifs (A, B and C).

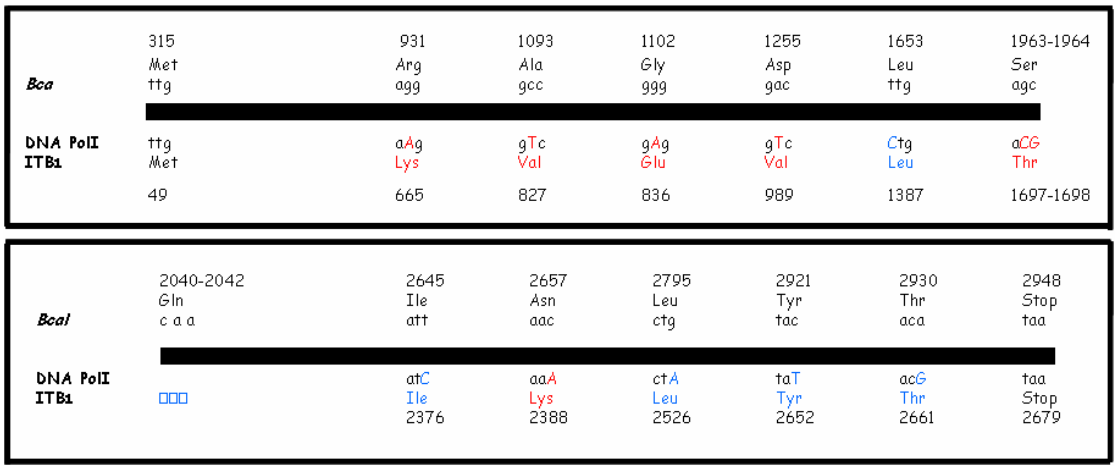

Figure 4. Detail comparison between the sequences of *Bca* DNA Pol1 and DNA Pol1 ITB$_1$. There are 15 nucleotide changes caused 6 amino acids substitution (red color) and 5 silent mutations (blue color). There is one amino acid (Gln) deletion in the sequence of DNA Pol1 ITB$_1$ (blue square).

5'→3'polymerase domain contain all motifs and important residues, including the six 5'→3'exonulease and the three 5'→3' polymerase motifs (Fig. 3 A,C). However, some important amino acid residues in the 3'→5' exonuclease domain were replaced by other (Fig. 3B). The deduced amino acid sequence of DNA Pol1 ITB$_1$ is closely related to those of bacterial DNA Pol1 in particular to *Bacillus* and *Geobacillus* DNA Pol1. DNA Pol1 ITB$_1$ showed 99.3% identity to *B. caldotenax* DNA polymerase (Accession No Q0457), 89.6% identity to *G. stearothermophilus* DNA polymerase (Accession No AAB52611), and 68.3% identity to *B. subtilis* DNA polymerase (Accession No NP390787). Detail comparison of DNA Pol1 ITB1 to *Bca* polymerase showed that there were 15 nucleotide changes caused one amino acid deletion in DNA Pol1 ITB$_1$, 6 amino acid substitutions, and 5 silent mutations (Fig. 4).

Expression and Purification of *DNA Pol1 ITB1*

DNA Pol1 ITB$_1$ was overexpressed in *E. coli* GI724 through pTRXFus expression vector. The DNA polymerase gene was amplified and inserted into *Kpn*I and *Bam*HI site of pTRXFus (Fig. 5). The resulted plasmid, namely pITB9, was then used to transform *E. coli* GI724. The culture of *E. coli* harboring pITB9 was induced by the addition of tryptofan. The cells were harvested and sonicated. DNA Pol1 ITB$_1$ was expressed as soluble form in cytosol. *E. coli* proteins were removed by heating at 80^{o}C for 10 min, however, some of *E. coli* proteins still remain. The soluble supernatant from heating step was then further purified using ammonium sulfate fractionation and anion exchange chromatography (DEAE). The purification of the enzyme was monitored by SDS-PAGE (Fig. 6). The SDS-PAGE revealed a single protein band with molecular mass of 110 kDa, which was in good agreement with the sum of molecular mass of DNA Pol1 ITB1 (99 kDa), calculated by deduced amino acid sequence, and 12 kDa of thioredoxine.

Polymerase Activity and Properties of *DNA Pol1 ITB1*

The polymerase activity of the enzyme was assayed by non radioactive method based on the technique developed by Kahler *et al.* (2000). The specific activity of the enzyme under the experimental condition as state on the methodology showed at 3.7unit/mg. The properties of the enzyme were determined, including the effect of pH and variation of temperature incubation. The dependence of the DNA Pol1 ITB$_1$ activity on the pH was determined under the pH range of 6.5 – 9.5. The buffer used was 50 mM TrishHCl. The maximum activity of the enzyme was showed at pH 7.5 (Fig. 7A). The dependence of DNA Pol1 ITB$_1$ activity on temperature was determined in the temperature range of 60 – 85^{o}C. The enzyme showed the highest activity at 65^{o}C (Fig. 7B).

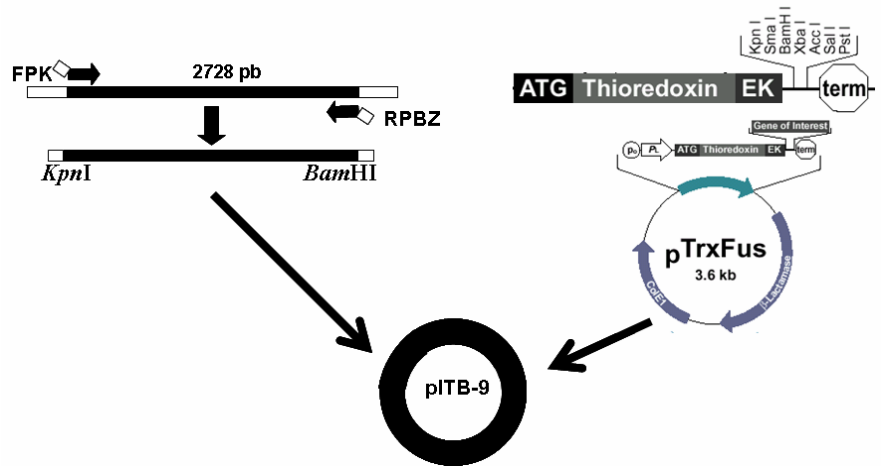

Figure 5. Construction of the recombinant plasmid pITB9.
The coding region of DNA Pol1 ITB_1 was amplified using FPK and RPBZ primers. The resulted fragment was introduced into an expression vector pTrxFus™ by using *Kpn*I and *BamH*I restriction site.

DISCUSSION

We have isolated and sequenced DNA polymerase I gene from thermophilic bacteria *G. thermoleovorans*. The gene was cloned using two steps of PCR amplification. There are very high conserved region in all DNA Pol1 from bacteria in the polymerase domain. Two region (around 5-7 amino acid residues) separated by around 200 amino acid residues in polymerase domain existed in most of DNA Pol1 sequences. These regions were used to construct the internal primer. The method used to clone *DNA POL1 ITB_1* was relatively simple since most of the approaches to clone DNA polymerase gene were used hybridization method to genomic library (Uemori *et al.,* 1993; Kim *et al.*, 2002; Shin *et al.*, 2005). In addition some techniques to clone DNA polymerase gene has been patented (Ishino *et al.*, 2003).

The nucleotide sequence of *DNA POL1 ITB_1* revealed that the start codon of the gene was TTG codon. This start codon is same as start codon from *Bca* polymerase from *B. cadotenax*. Uemori *et al.* (1993) have been analyzed using deletion mutants and proved that *Bca* polymerase start codon was TTG codon. In addition to polymerase activity, DNA Pol1 also exhibit 5'→3' exonuclease or 3'→5' exonuclease or both activities (Perler *et al.*, 1996). In *E coli* there are 3 separate domains which are responsible for those activities. Each domain consist unique motifs (Joyce *et al.*, 1994). Homological analysis of DNA Pol1 ITB_1 showed that the protein consist the 3 separate domains.

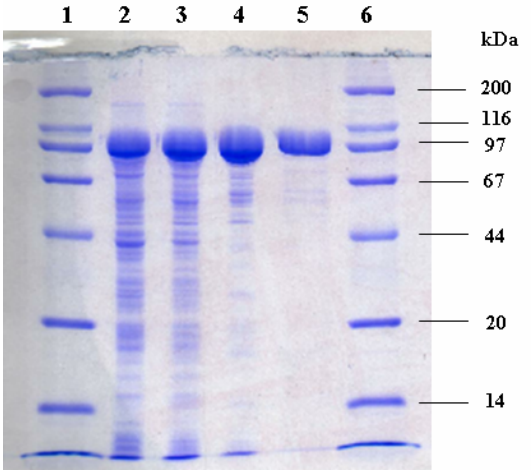

Figure 6. SDS-polyacrylamide gel electrophoresis of the enzyme during purification of the DNA polymerase. The SDS-10% polyacrylamide gel was stained by Coomassie blue R-250 after electrophoresis. Lane 1 and lane 6, broad-range marker (Bio-Rad); lane 2, sonicated crude extract ; lane 3,crude extract after heat treatment (10 min at 80°C) ; lane 4, protein fraction from ammonium sulphate fractionation (20-40%); lane 5, protein purification eluted from anion exchange chromatography (DEAE).

The 5'→3' exonuclease and 5'→3' polymerase domains contained all of conserved amino acid residues, while the 3'→5' exonuclease domain did not contain amino acid residues which are responsible for 3'→5' exonuclease activity. The phenomena also revealed on DNA polymerase

from the genus of *Bacillus* and *Geobacillus*. The 5'→3' exonuclease domain consists 6 motifs (Fig. 3A), there were six carboxylates residues in DNA Pol1 ITB_1 which are identical to corresponding residues in Taq polymerase which proved to have role on 5'→3' exonuclease activity (Kim *et al.*, 1995). There are 3 motifs, Exo I, II, and III, in the 3'→5' exonuclease domain of DNA polymerase (Blanco *et al.,* 1992), these motifs was shown by four carboxylate residues, 1 aspartate and 1 glutamate residues on Exo I, 1 aspartate on ExoII, and 1 aspartate on Exo III. The aspartate residue on Exo I was replaced by valine residues while aspartate on Exo II and Exo III were replaced by alanin and lysine residues in DNA Pol1 ITB_1 (Fig. 3B). The amino acid replacement also occurred on other DNA pol1 which known do not exhibited 3'→5' exonuclease activity. The DNA Pol1 ITB_1 consist six 5'→3' polymerase motifs (data not shown) as known for Pol1 from bacteria (Patel *et al.*, 2001). Three motifs (Fig. 3C), A, B, and C, consists carboxylate triad which known has a key role on polymerase activity (Kiefer *et al.*, 1997; Patel *et al.*, 2001). From all of these data suggested that DNA Pol1 ITB_1 exhibited the 5'→3' exonuclease and 5'→3' polymerase activities, while the 3'→5' exonuclease domain is possibly as vestigial region.

A few moderate thermostable polymerases have been isolated and characterized from the genus of *Bacillus* (Perler *et al.*, 1996). *Bca* polymerase from *Bacillus caldotenax* has been cloned, and purified (Uemori *et al.*, 1993). The protein has the highest similarity with DNA Pol1 ITB_1, the molecular weight of this protein was reported at around 99 kDa in size which was similar to DNA Pol1 ITB1 (Fig. 6). Sellmman *et al.* (1992) isolated and characterized DNA pol1 from *B. stearothermophilus*. This polymerase has optimum activity at around 65°C. The fusion protein of DNA Pol1 ITB_1 with 12 kDa of thioredoxine still showed polymerase activity with the optimal condition at 65°C and pH 7.5, this is in agreement with all known DNA Pol1 from the genus of *Bacillus* and *Geobacillus* (Akhmaloka *et al.*, 2006; Perler *et al*., 1996).

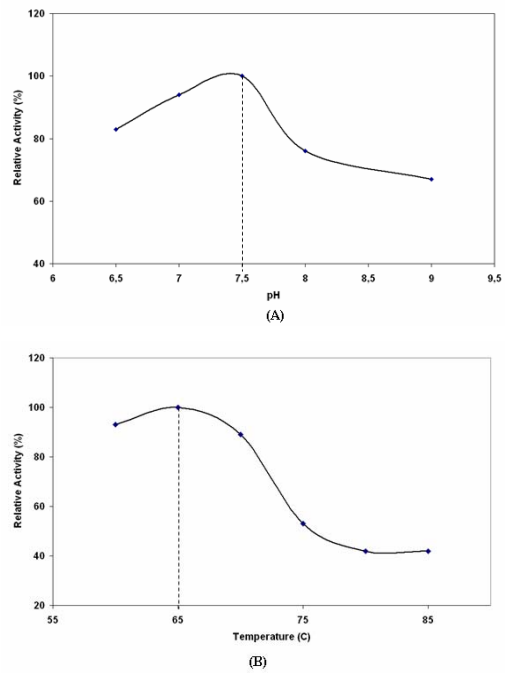

Figure 7: The dependence of the polymerase activity on pH and temperature. (A). Effect of pH on the activity of DNA Pol1 ITB_1 (B). Effect of temperature on the activity of DNA Pol1 ITB_1

Acknowledgement

This report was supported by the Competitive Research Grant from Ministry of National Education and Riset Unggulan Terpadu from Ministry of Research and Technology, Republic of Indonesia.

References

Akhmaloka, Tika, I N. *et al.* (2006) Isolation and characterization of a novel thermostable DNA polymerase from *Bacillus thermophilus* A1. *Indonesian Journal for Microbiology*. **11**: 82-86.

Blanco, L., A., Bernad, M. A. *et al.* (1991). A general structure for DNA-dependent DNA polymerases. *Gene* **100:** 27-38.

Blondal, T., Thorbjarcidsnardottir, S.H. *et al.* (2001). Cloning, Sequence Analysis and Functional Characterization of DNA Polymerase I from the Thermophilic Eubacterium *Rhodothermus marinus*. *Biotechnol. Appl. Biochem*. **34**:37 – 45.

Cariello, N. F., Swenberg, J.A. *et al.* (1991). Fidelity of *Thermococcus litoralis* DNA polymerase (Vent) in PCR determined by denaturating gradient gel electrophoresis. *Nucleic Acids Res.* **19**: 4193-4198.

Chien, A., Edgar D.B. *et al.* (1976). Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. *J. Bacteriol.* **127:** 1550-1557.

Choi, J. J. and Kwon S. T. (2004). Cloning, expression, and characterization of DNA polymerase from hyperthermophilic bacterium *Aquifex pyrophilus*. *J. Microbiol. Biotechnol.* **14:** 1022-1030.

Frey, B. and Suppmann, B. (1995). Demonstration of the Expend PCR Systems greater fidelity and higher fidelity and higher yields with a lacI-based fidelity assay. *Biochemica*. **2**: 34-35.

Indrajaya, Madayanti F. *et al.* (2003). Isolation and Identification of Thermophilic Microorganism from Wayang Crater. *Indonesian Journal for Microbiology*. **8**: 53-56.

Ishino, Y., Uemori, T. *et al.* (2003). Method for cloning of a gene for pol I type DNA polymerase. *Patent No EP517418*.

Joyce, C.M., and Steitz, T.A. (1994). Function And Structure Relationships In DNA Polymerases, *Annu. Rev. Biochem*. **63 :** 773 – 822

Kaboev, O.K., Luckia L. K. *et al.* (1981). Purification and properties of deoxynucleic acid polymerase from *Bacillus stearothemrophilus, Biokhimia.* **45**: 664-651

Kähler, M. and Antranikian G. (2000). Cloning and characterization of a family B DNA polymerase from the hyperthermophilic crenarchaeon *Pyrobaculum islandicum*. *J. Bacteriol.* **182:** 655-663.

Kiefer, J. R., Mao, C. *et al.* (1998). Visualizing DNA replication in a catalytically active *Bacillus* DNA polymerase crystal (see comments). *Nature*. **391**: 304-307.

Kim, Y. J., Lee, H. S. *et al.* (2007). Cloning, purification, and characterization of a new DNA polymerase from a hyperthermophilic archaeon, *Thermococcus sp.* NA1. *J. Microbiol. Biotechnol.* 17: 1090-1097.

Kim, D. J., Jang, H. J. *et al.* (2002). Cloning, Expression, and Characterization of Thermostable DNA Polymerase from *Thermoanaerobacter yonseiensis*. *Journal of Biochemistry and Molecular Biology.* **35**: 320-329.

.

Kim, Y., Eom, S. H. *et al.* (1995). Crystal structure of Thermus aquaticus DNA polymerase. *Nature*. **376**: 612-616.

Kornberg, A. and Baker K. (1992). *DNA Replication*, 2nd Ed. Freeman and Company, New York, U.S.A.

Lundberg, K. S., Shoemaker D. D. *et al.* (1991). High-fidelity amplification using a thermostable DNA polymerase isolated from *Pyrococcus furiosus*. *Gene* **108:** 1-6.

Mullis, K., Faloona, F. *et al.* (1986). Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symp. Quant. Biol.* **247**: 7116-7122.

Patel, P. H., Kawate, H. *et al.* (2001). A single highly mutable catalytic site amino acid is critical for DNA polymerase Fidelity. *J. Biol. Chem.* **276**: 5044-5051.

Perler, F. B., Kumar S. *et al.* (1996). Thermostable DNA polymerases. *Adv. Protein Chem.* **48**: 377-435

Saiki, R. K., Gelfand D. H. *et al.* (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science.* **239:** 487-491.

Sambrook, J., Fritsch E. F. *et al.* (1989). *Molecular Cloning: A Laboratory Manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, New York, U.S.A.

Sellman, E., Schroder K. L. *et al*. (1992). Purification and characterization of DNA Polymerase from *bacillus* species, *J. Bacteriol*. **174**: 4350-4355

Shin, H.-J., S.-K. Lee S. K. *et al.* (2005). Cloning, expression, and characterization of a family B-type DNA polymerase from the hyperthermophilic crenarchaeon Pyrobaculum arsenaticum and its application to PCR. *J. Microbiol. Biotechnol.* **15**: 1359-1367.

Steitz, T. A. (1999). DNA polymerases: structural diversity and common mechanisms. *J. Biol. Chem.* **274**: 17395-17398.

Stenesh. J and Roe R. A. (1972). DNA Polymerase from mesophilic and thermophilic bacteria. I. Purification and properties of DNA Polymerase rom *Bacillus licheneformis* and *Bacillus stearothermophilus, Biochem. Biophys. Acta*. **272**: 156-166

Uemori, T., Ishino Y. *et al.* (1993). Cloning of the DNA polymerase gene of *Bacillus caldotenax* and characterization of the gene product. *J. Biochem*. **113**: 401-410