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Cloning, Expression, and Characterization of Thermostable DNA Polymerase from *Thermoanaerobacter yonseiensis*

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A gene, coined *tay*, for a thermostable DNA polymerase from the novel, extremely thermophilic bacterium *Thermoanaerobacter yonseiensis* was cloned and expressed in *E. coli*. Using a DNA polymerase homologous PCR product as a hybridization probe, *tay* was isolated and sequenced to consist of 2616 nucleotides that encode 872 amino acids. A database analysis showed that DNA polymerase, coined *Tay*, from *T. yonseiensis* shared a 39% to 47% identity in the amino acid sequence with those from other DNA polymerases. *Tay* was overexpressed in *E. coli* as a fusion protein with a poly-histidine tag at the C-terminus. It was purified by heat treatment, followed by a Ni^{2+}-chelate column. The molecular weight of purified *Tay* was approximately 97 kDa, as shown by SDS PAGE, and it showed high DNA polymerase activity and thermostability. However, it had no 3'→5' exonuclease activity.

Keywords: *Thermoanaerobacter yonseiensis*, Thermostable DNA polymerase.

Introduction

DNA polymerases are key enzymes in the replication of cellular information that is present in all living things. They are a family of enzymes that are involved in DNA replication and repair.

Beginning with the isolation and characterization of DNA polymerase I from *Escherichia coli* by Kornberg and colleagues in the 1950s (Kornberg, 1980), more than 50 DNA polymerases have been cloned and sequenced from various organisms, including thermopile and archaea. DNA polymerases have been used extensively in molecular biology. For example, DNA polymerase I was used for the nick-translation of DNA and synthesis of the second strand of cDNA in cDNA cloning (Efstratiadis *et al.*, 1976). The modified version of bacteriophage T7 DNA polymerase (Sequenase™) was used for the dideoxy sequencing of DNA (Tabor and Richardson, 1987). Thermostable DNA polymerase, such as *Taq* DNA polymerase, has been the key element in the development of the polymerase chain reaction (PCR) (Saiki *et al*., 1988; Mullis *et al*., 1986). PCR was also performed using the Klenow fragment of *E.coli* DNA polymerase I. However, it needs to be added every cycle after the denaturation and primer hybridization steps since this enzyme is heat-sensitive. The application of thermostable DNA polymerase in PCR makes the automation of PCR possible. *Taq* DNA polymerase from *Thermus aquaticus* was the first characterized thermostable enzyme (Chien, 1976). Thereafter, many DNA polymerases from the *Thermus* strain were studied. *Tfl*, *Tth*, *Tfi*, and *Top* polymerase have been applied to PCR (Kaledin *et al*, 1981; Ruttimann *et al*, 1985; Jung *et al*., 1997; Kim *et al*., 1998); however, their base-insertion fidelity is low because these DNA polymerases do not have 3'→5' exonuclease activity. The high-fidelity of DNA polymerases, which has 3'→5' exonuclease-dependent proofreading activity, should be required for error correction during the polymerization. Several thermostable DNA polymerases with proofreading activity (*Pfu*, *Vent*, *deep Vent*, and *Pwo*) have also been studied and introduced for high-fidelity PCR amplification (Cariello *et al*., 1991; Lunberg *et al*., 1991; Kong *et al*., 1993; Frey and Suppmann, 1995; Cline *et al*., 1996).

Most of the native thermostable enzymes are synthesized at very low levels by the thermophilic bacteria; therefore, they are cumbersome to purify. So thermostable DNA polymerase, such as *Pfu* DNA polymerase, was produced in a biologically active form in the *E.coli* over-expression system (Lu and Erickson, 1997; Dabrowski and Kur, 1998). However, several problems persist, such as error-prone amplification and unwanted amplification at low temperatures in the PCR applications. New and improved thermostable DNA polymerases are needed.

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In this report, we describe the discovery of a gene that encodes a novel DNA polymerase from thermoanaerobic bacteria, *Thermoanaerobacter yonseiensis KB-1* (Kim *et al.*, 2001). This organism is known as one of the most heat-resistant bacteria. Enzymes from this bacteria showed a higher heat-stability (Jang *et al.*, 2002). The thermostable DNA polymerase (*Tay*) from the cloned gene was over-expressed in *E. coli* and characterized.

Materials and Methods

Preparation of the *T. yonseiensis KB-1* genomic DNA library *T. yonseiensis* genomic DNA was partially digested with *Sau*3AI. The fragments (lengths, 2-12 kb) were ligated into the ZAP EXPRESS vector / *Bam*HI (Stratagene, N. Torrey Pines Road, La Jolla, USA). They were packaged using a Gigapack III Gold packaging extract (Stratagene, La Jolla, USA).

Preparation of the homologous primer and PCR From an amino acid sequence alignment of known bacterial DNA polymerases, a highly conserved sequence was selected in order to make a probe. Degenerate primers were designed, based on the conserved sequence. The sense primer was 5' CC(AGCT)-AA(CT)-(CT)T(AGCT)-CA(AG)-AA(CT)-AT(ATC)-CC(AGCT) 3'. The antisense primer was 5' (AGCT)A(AG)-(CT)TC-(AG)TC-(AGCT) A(CT)-(CT)TG 3'. A polymerase chain reaction (PCR) was performed in a total volume of 50 μl using the PCR system 2400 (Applied Biosystems, Foster City, USA). The mixture contained 0.5 unit of *Taq* DNA polymerase, 5 μl of 10 × *Taq* DNA polymerase buffer, 0.2 mM of each dNTP, 100 pmole of each primer, and 0.5 μg of *T. yonseiensis* genomic DNA. PCR was performed by 30 amplification cycles on the condition, denaturation (96°C, 2 min), annealing (46~51°C, 1 min), and extension (72°C, 30 s). After the final cycle, the reaction mixture was kept at 72°C for 7 min.

DNA probe preparation The PCR product was detected with ethidium bromide staining on 1% agarose gel and cloned into a pGEMT-easy vector (Promega, Madison, USA). The plasmid that contained the PCR product was prepared for the preparation of more probes and sequenced to confirm. For screening of the *T. yonseiensis* genomic (DNA) library, the probe was labeled with [α-^{32}P]dCTP using the Prime-a-Gene labeling system.

Screening of the *T. yonseiensis* genomic (DNA) library with isotope labeled probe. To locate λ that contained the DNA polymerase gene, λ plaques were lifted onto Hybond N+ membranes (Amersham Pharmacia Biotech, Uppsala, Sweden), lysed, then hybridized with [^{32}P]-labeled probe. The plasmids were rescued according to the protocol of the ZAP Express® predigested Gigapack cloning kit (Stratagene, N. Torrey Pines Road, La Jolla, USA) into a pBK-CMV phagimids' vector using the Ex-Assist® helper phage. This plasmid was digested with *Apa*I and *Bam*HI to remove the insert. It was analyzed on 1% agarose gel in a Tris-acetate-EDTA (TAE) buffer.

Sequence analysis Sequences of the double-stranded plasmid DNA were determined using a Dye Terminator Cycle Sequencing Ready mixture (Applied Biosystems, Foster City, USA) on a PCR system 2400 and ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). To sequence the DNA polymerase gene, the cloned plasmid was used as a source of the template DNA. Initial sequencing was performed using the T7 and SP6 primers as the primers. When necessary, additional primers were then synthesized as needed, using newly obtained sequence data. Sequence comparisons were done using the BLAST program (Altschul *et al.*, 1990).

Inverse PCR for screening In order to isolate the part of the gene that encodes the DNA polymerase, inverse PCR was performed (Silver and Keerikatte, 1989). Three μg of the *T. yonseiensis* total DNA was cut with *Hin*dIII. This DNA was diluted (1/10) and self-ligated to form circles. The proper-size DNA circles were isolated and amplified by PCR using two primers, designed according to the known DNA sequence from the first isolate. Sequences of the primers were as follows: sense primer (T3-3), 5'-CCTTAAAGTGGATGTATTGTCCC-3'; antisense primer (T7-4), 5'-CTTTGTAAGAAAGTTTCACCAGG-3'. DNA amplification was performed using 2.5 units of *Pfu* DNA polymerase (Stratagene, N. Torrey Pines Road, La Jolla, USA) in a 50 μl reaction mixture, which included 10 × PCR reaction buffer, 20 pmol of each primer, 0.2 mM of each dNTP, and 0.2 μg of circular DNA. Prior to the reaction cycle, the mixture was heated to 96°C for 3 min, followed by 30 cycles at 96°C for 30 sec, 50~53°C for 30 sec, 72°C for 4 min, then the final extension at 72°C for 7 min. After the final extension, 2.5 units of *Taq* DNA polymerase was added to the reaction mixture and incubated at 72°C for 5 min. The amplified 1.5 kb PCR product was identified on 1% agarose gel and subcloned into a pGEMT-easy vector (Promega, Madison, USA). This plasmid was sequenced by an automatic sequencer ABI310 (Applied Biosystems, Foster City, USA) in the same manner, as described previously.

Computer analysis The computer-assisted DNA and protein sequence analysis were performed using the DNASIS version 7.0 and PROSIS. The BLAST program at the NCBI server was used to search for sequence similarity.

Construction of the expression plasmid For the expression of the *Tay* DNA polymerase, four primers were designed. The first set, 5' end primer (Ex-k-6, 5' GGGAATTCCATATGGCAAAGTTTCT GTTAATTGATGGTAGCAG3') that contained the *Nde*I restriction enzyme site (under-lined) and 3' end primer (T7-4, 5' CTTTGTAA GAAAGTTTCACCAGG 3') was designed according to the sequence of the 1.5 kb inverse PCR product. The second set, 5' end primer (Ex-2-1, 5' GCATAGAATGTAAGAGTAAATATC 3') and 3' end primer (Ex-2, 5' CCGCTCGAGCTTAGCCAAAAACCAG TTAGG 3') that contained the *Xho*I restriction enzyme site at the end position (under-lined) was designed according to the sequence of 2.6 kb DNA that was identified first. The T7-4 and Ex-2 primers were in fact designed according to the 170 bp DNA sequence that overlapped between the two isolates. DNA amplification was performed using two primers (Ex-k-6 and T7-4) on a 1.5 kb inverse PCR product as a template: 30 cycles at 96°C for 30 s, 58°C for 30 s, 72°C for 1 min and 30 s, and 1.1 kb PCR product that was eluted from 1% agarose gel by the Gene-clean method (Bio101,

Carlsbad, USA). Four cycles of the PCR reaction were performed using 1.1 kb DNA that was eluted from the gel and 2.6 kb first-isolated DNA as the mega-primer and template, respectively. Thirty amplifications were performed on the condition, denaturation (96°C, 30 s), annealing (58°C, 30 s), and extension (72°C, 3 min). After the final cycle, two additional primers(Ex-k-6, Ex-2) that contained the *Nde*I and *Xho*I restriction enzyme site, respectively, were added to the reaction mixture. These continued for 30 cycles of amplification under the following conditions: denaturation (96°C, 30 s), annealing (58°C, 30 s), and extension (72°C, 5 min). The 2.6 kb amplified PCR product was identified on the 1% agarose gel. The PCR product was eluted and digested with *Nde*I and *Xho*I. The pET22b (+) expression vector (Novagen, Madison, USA), containing the sequence that encoded the C-terminal 6× His affinity tag, was digested with *Nde*I and *Xho*I in advance. The 2.6 kb PCR product and vector were ligated with T4 DNA ligase (Promega, Madison, USA) at 16°C overnight. The recombinant plasmid was introduced into *E.coli* DH5α by the heat-shock method. The cloned 2.6 kb gene, coined *tay*, was identified by sequencing in the same manner, as described previously.

Expression and purification of the *Tay* DNA polymerase Protein, coined *Tay*, was expressed in *E.coli* BL21(DE3)pLysS. A single colony of *E. coli* BL21(DE3)pLysS that contained the recombinant DNA was picked. It inoculated 3 ml of LB broth that contained 50 μg of ampicillin per ml. Then it was incubated at 37°C overnight. The overnight culture was transferred to 200 ml LB medium that contained 50 μg of ampicillin per ml. The culture was incubated until OD_{600} = 0.5, then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), followed by further incubation for 3 h. The cells were harvested by centrifugation (5,000 × *g* at 4°C for 10 min) and resuspended in 10 ml binding buffer (20 mM Tris-HCl pH 7.9, 0.5 M NaCl, 5 mM imidazole). The cells were sonicated five times for 30 s each time with 30 s intervals. The cell debris was removed by centrifugation (15,000 × *g* at 4°C for 30 min). The supernatant was transferred to a 15 ml conical tube and incubated in a 80°C water bath with shaking for 10 min. The heat-treated supernatant was cooled on ice for 20 min, then centrifuged (17,000 × *g* at 4°C for 30 min) to remove the denatured *E. coli* proteins. A His-bind resin and His-bind buffer kit (Novagen, Madison, USA) were used to purify the His-tagged protein, according to Novagens' manufacturers' instructions. The protein was eluted from resin with 3 ml of the elution buffer (20 mM Tris-HCl pH 7.9, 10 mM imidazole, 0.5 M NaCl), 0.5 ml per fraction. The protein concentration was measured according to Bradford (1976). The active fractions were pooled and dialyzed against the storage buffer (20 mM Tris-HCl pH 7.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT) at 4°C overnight.

DNA polymerase assay The *Tay* DNA polymerase activity was measured by the modified assay method that was described previously (Bohlke *et al.*, 2000). The method was based on the incorporation of [α-^{32}P]dCTP in a DNA template (M13mp18 ssDNA). A 50 μl reaction mixture that contained 5 μl 10× *Taq* reaction buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 200 μM each of dATP, dGTP, dTTP, dCTP, 1 μCi [α-^{32}P]dCTP, enzyme solution (1~10 μl), and 1 μg of M13mp18 ssDNA was annealed with 0.3 μg of (–20) forward primer. Assay reactions were incubated at 72°C for 30 min, stopped on ice by the addition of 250 μl 10% TCA, and kept on ice for 10 min. The samples were filtered through GF/C filters (Whatman, Maidstone, UK). The filters were washed three times with 50 ml of 5% TCA, 20 mM sodium pyrophosphate, then washed with 70% ethanol. The washed filters were air-dried and counted by a liquid scintillation counter (Packard Bioscience, Billerica, USA). *Taq* DNA polymerase was used as a positive control. One unit is defined as the amount of enzyme that is necessary to incorporate 10 nmol dNTP into an acid-insoluble form at 72°C in 30 min.

Effect of pH, metal ion, dNTP concentration, and temperature on *Tay DNA* polymerase activity The recombinant *Tay* DNA polymerase was investigated for optimal reaction conditions using DNA polymerase assay, as described previously. The activity of *Tay* was measured in different conditions; pH, $MgCl_2$ concentration, and KCl concentration (Niehaus *et al.*, 1997). The effect of the dNTP concentration on the activity of *Tay* was analyzed using 12% polyacrylamide gel electrophoresis. The synthetic primer (Ex-kexo, 5' CCATCAATTAACAGAAACTTTGC 3') and the synthetic template (Ex-k, 5' GGGAATTCCATATGGCAAAGTTTCTGTTA ATTGATGG 3') were used in this assay. DNA polymerase activity of *Tay* was measured using 5'-[^{32}P]-labeled-primer and labeled primer/template hybrid. One pM to 10 μM dNTPs and 0.02 unit of *Tay* were incubated in a 10× *Tay* buffer (10 mM Tris-HCl pH 8.0, 90 mM KCl, 2 mM $MgCl_2$, reaction volume 25 μl) at 75°C for 30 min. The reaction was stopped with 5 μl formamide buffer on ice. The samples were analyzed by 12% polyacrylamide sequencing gel with 7 M urea in a TBE buffer (1500 V, 2 h) and visualized by autoradiography.

Fidelity assay In order to investigate the fidelity of *Tay* DNA polymerase, gapped M13mp18 DNA was used as substrate (Campbell, 1995). Single- and double-stranded DNA were purified from a M13mp18 phage particle and *E. coli*, respectively (Sambrook *et al*, 1989). To obtain *lacZ* site gapped DNA, 2.7 μg of the RF form of double-strand M13mp18 DNA was digested with *Hin*dIII and *Bgl*II. A 650 bp DNA fragment that encoded the *lacZ* region and 6.6 kb rest of the DNA were separated on 1% agarose gel. The 6.6 kb DNA that was eluted from the gel was denatured at 90°C for 5 min, and the same molar ratio of single-strand M13mp18 DNA was added. The mixture was placed on ice for 5 min, then SSC (300 mM NaCl, 30 mM sodium citrate) was added to the final 2× concentration. The mixture was incubated at 60°C for 5 min, then placed on ice. The hybridized DNA was precipitated with ethanol and resuspended in a TE buffer. The single-stranded DNA was converted to double-stranded circular DNA that contained 650 bp gap at the *lacZ* site. DNA synthesis reaction was performed using a thermo cycler in a total volume of 25 μl. The reaction mixture contained 0.02 unit of *Tay*, 2.5 μl of 10× *Tay* buffer, and 100 ng of gapped DNA. The DNA synthesis reaction was incubated at 75°C for 10 min and stopped on ice by the addition of 4 mM EDTA. Gap-filled DNA was introduced to the *E. coli* XL-1Blue MRF competent cell. The transformed cell was added to a tube at 48°C, containing 3 ml of soft agar, 500 μg of IPTG, and 2.5 mg of X-Gal. The soft agar that contained the transformed cell was poured onto the M9 minimal plate and solidified. The plate was incubated overnight at 37°C, then scored for the mutant.

Results and Discussion

Cloning and sequencing of the *tay* gene from *T. yonseiensis*

The PCR reaction with the primers that were designed with the homologous region of DNA polymerases was successfully performed on the DNA λ library that was prepared from the *T. yonseiensis* DNA. The PCR product was cloned in a pGEMT-easy vector and sequenced. The sequence of the PCR product was homologous with those of DNA polymerases from other bacteria (data not shown). Using this PCR product as a probe, the genomic DNA library of *T. yonseiensis* was screened. The positive spot was cloned to the pBKCMV phagimid vector and sequenced. A sequence analysis of the 2.6 kb DNA from the positive clone contained only a part of the 3' end of the gene encoding DNA polymerase. In order to find the rest of the gene, inverse PCR was performed. The 1.5 kb PCR product was obtained and sequenced. It contained the other parts of the DNA polymerase gene. The 1.5 kb DNA region from the 5' end of the 2.6 kb first isolate was homologous with those of DNA polymerase in other bacteria, whereas the 1.1 kb DNA region from the 3' end of the 1.5 kb inverse PCR product was homologous with them. Two segments of the

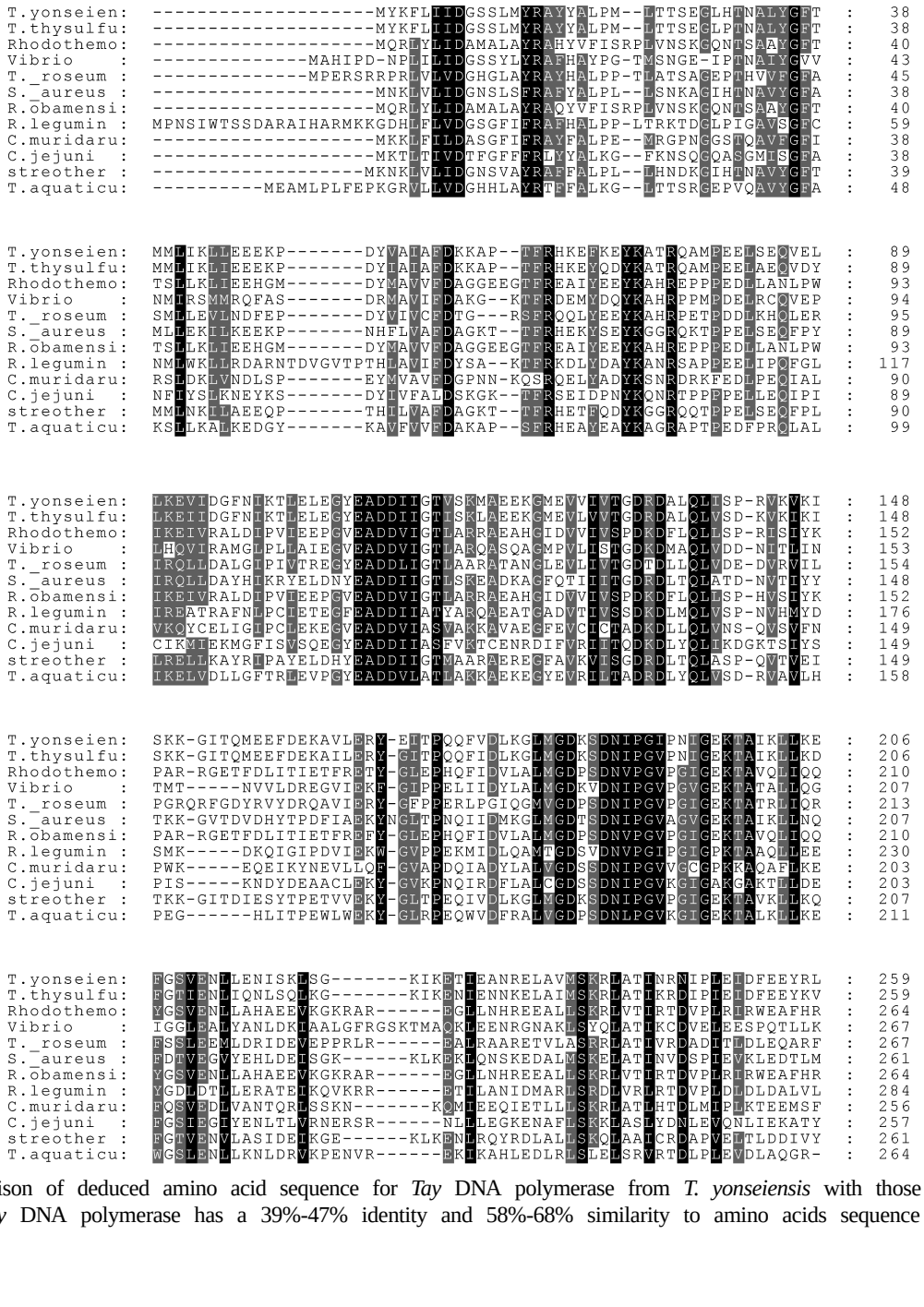

Fig. 1. Comparison of deduced amino acid sequence for *Tay* DNA polymerase from *T. yonseiensis* with those of other DNA polymerases. *Tay* DNA polymerase has a 39%-47% identity and 58%-68% similarity to amino acids sequence of other DNA polymerases.

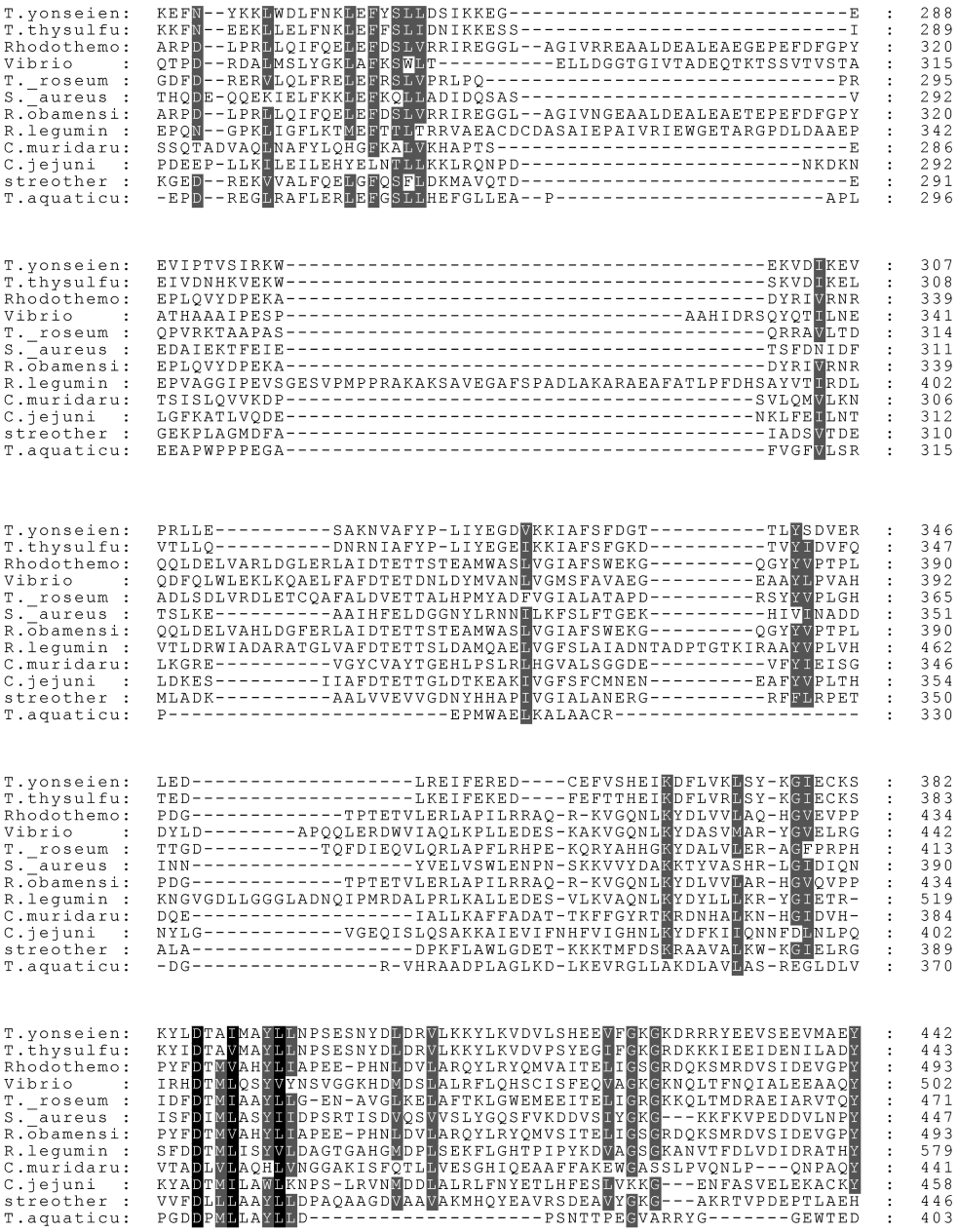

Fig. 1. Continued.

homologous DNAs were connected by PCR and sequenced. The connected gene, coined *tay*, has 2616 nucleotides and 872 deduced amino acids. The complete nucleotide sequence of *tay* was previously deposited at the GenBank database under the access number AY065997. The BLAST program analyzed the sequence of the tay gene. A deduced amino acid sequence of the *tay* gene has a 58-68% homogeneity and 39-47% identity with those of DNA polymerase in other bacteria. The linear alignment of the amino acids from these genes is given in Fig. 1, showing the conserved domains.

Expression and purification The pET system (Novagen, Madison, USA) is one of the most powerful recombinant protein expression systems in *E. coli*. The pET22b(+) vector has a very strong T7 promoter and can be used in combination with pLysS to provide additional stringency (Dubenforff and Studier, 1991; Moffat and Pfaffle, 1995). The pLysS plasmids, which express the T7 lysozyme in the bacterial cytoplasm, strongly repress the protein expression from the pET vector in the absence of induction, thus enabling the expression of very toxic proteins. The *tay* gene was amplified using *Pfu* DNA polymerase and cloned into the *Nde*I site of pET22b (+), according to previously mentioned methods. The cloned *tay* gene was transformed into *E. coli* BL21(DE3)pLysS at 37°C. The selected colony was grown in a LB medium that was supplemented with ampicillin. Overexpression was induced

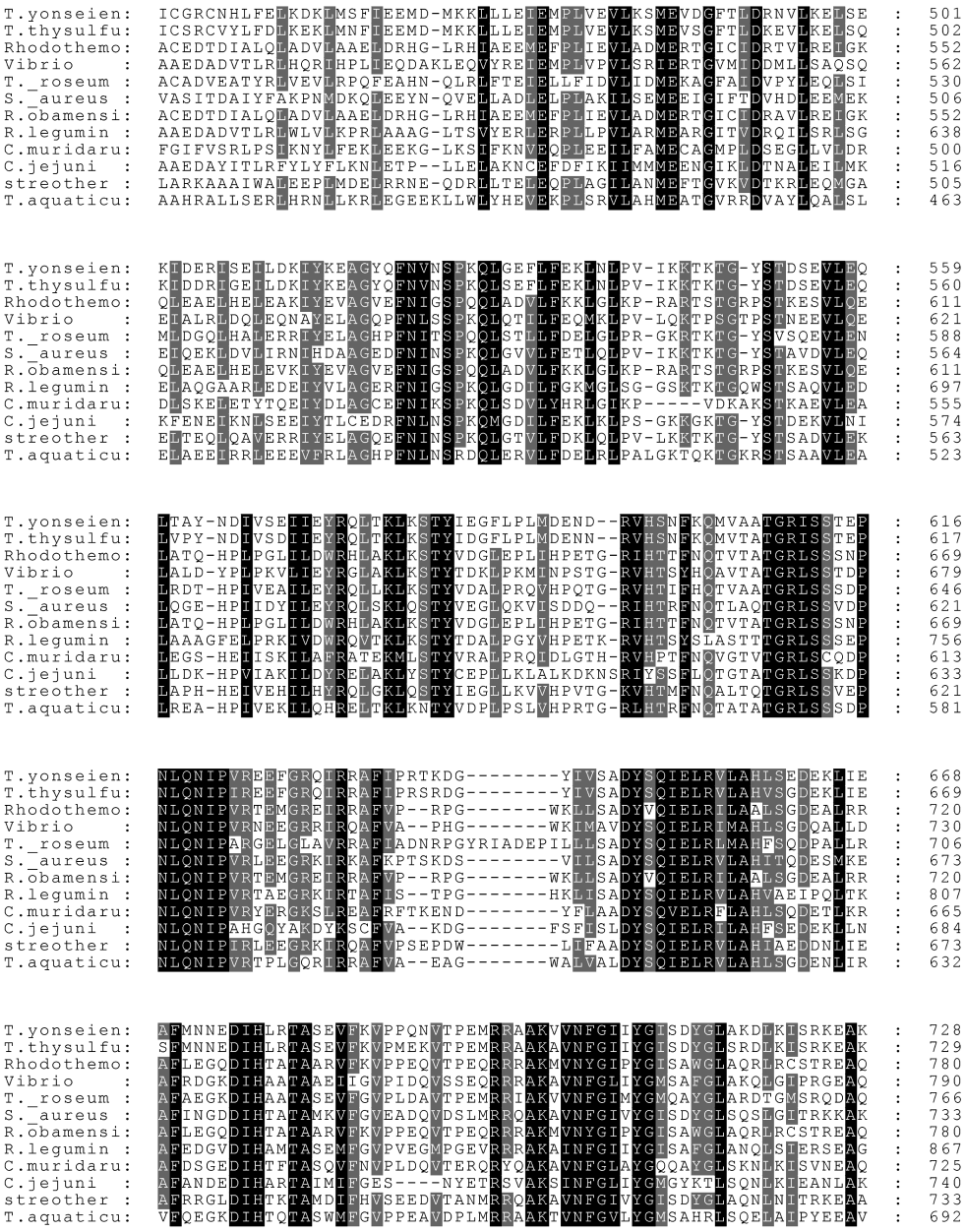

Fig. 1. Continued.

by the addition of IPTG (final concentration 1 mM). After a 3 h induction by IPTG, the cell was harvested and sonicated. Most of the *E. coli* proteins were denatured and precipitated by heat treatment at 80°C for 10 min, but some of the *E. coli* proteins remained soluble after heating (Niehaus *et al*, 1997). The soluble supernatant (after the heating step) was then purified with a Ni-NTA affinity column. Table 1 gives an overview of the purification procedure. The eluted protein was run in 10% SDS-PAGE (Fig. 2). The apparent molecular mass of protein was a 97 kDa, which correlated well to the predicted size from the nucleotide sequence data. The DNA polymerase activity of the purified protein, *Tay*, was measured using the modified acid precipitation method. One unit of *Tay* was defined as the amount of protein that catalyzed the incorporation of 10 nmol dNTP into an acid-insoluble form at 75°C for 30 min. After dialysis of *Tay* against the storage buffer, the DNA polymerase activity was 536 unit/mg for the purified His-tagged *Tay* DNA polymerase that was isolated from 200 ml *E. coli* culture.

Effect of pH, metal ion, dNTP concentration, and temperature on the activity of the *Tay DNA* polymerase. Characteristics of the *Tay* DNA polymerase (such as optimum pH, temperature, and required concentration of metal ion and dNTP) were determined. The effects of pH on the activity of *Tay* with three different buffers were used. *Tay* showed

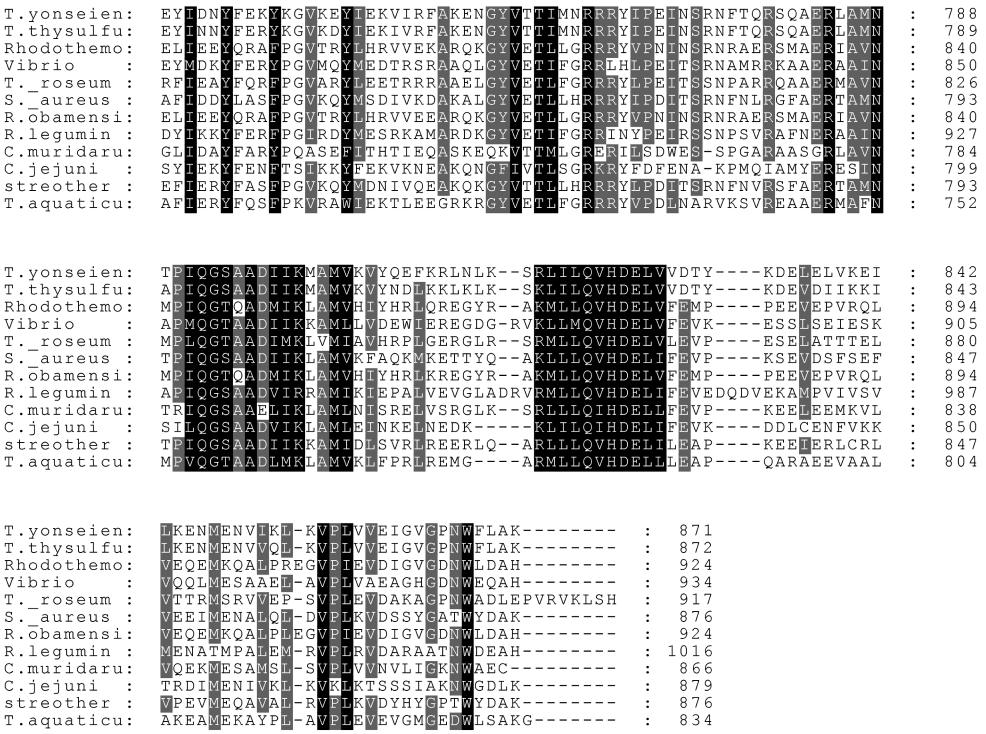

Fig. 1. Continued.

Table 1. Purification of recombinant *Tay* DNA polymerase

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
Crude extract	38.64	1371.33	35.49	100
Heat-treatment	9.99	744.05	74.48	54.26
His-resin	0.45	241.37	536.38	17.60

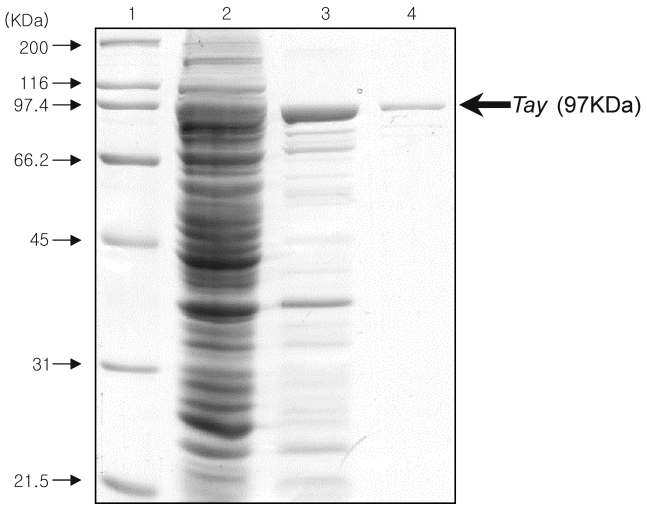

Fig. 2. SDS-PAGE analysis of *Tay* DNA polymerase. Lane 1, Size Marker; Lane 2, Lysate from *E.coli* BL21(DE3)pLysS carrying the vector with *tay* gene; Lane 3, Heat (80°C, 10 min) treated lysate from *E.coli* BL21(DE3)pLysS carrying the vector with *tay* gene; Lane 4, *Tay* purified from His-Bind resin.

maximum activity at the Tris-HCl buffer, pH 8.0 (Fig. 3A). Like all of the other DNA polymerase, *Tay* required divalent cation as a cofactor. Optimal activity was obtained with 2.5 mM $MgCl_2$ (Fig. 3B). The optimal concentration of KCl is 90 mM (Fig. 3C). The effect of temperature on the catalytic activity of the *Tay* was shown (Fig. 3D) to be optimum between 70~80°C. The polymerase activity of *Tay* was shown at a dNTP concentration over 100 nM (Fig. 4). In order to identify the 3'→5' exonuclease activity (proofreading activity) of *Tay* DNA polymerase, a 12% polyacrylamide sequencing gel was used. A 5'-[^{32}P] labeled-primer and primer/template synthetic oligonucleotide were used as a substrate in this assay. Under these artificial conditions, both the single- and double-stranded DNA were not degraded (Fig. 4). This indicates that the *Tay* DNA polymerase has no 3'→5' exonuclease activity.

Fidelity for *Tay* DNA polymerase Polymerization errors are rare events relative to correct incorporations. Highly sensitive assays are required to measure DNA polymerase fidelity. In order to detect the fidelity of *Tay* DNA polymerase,

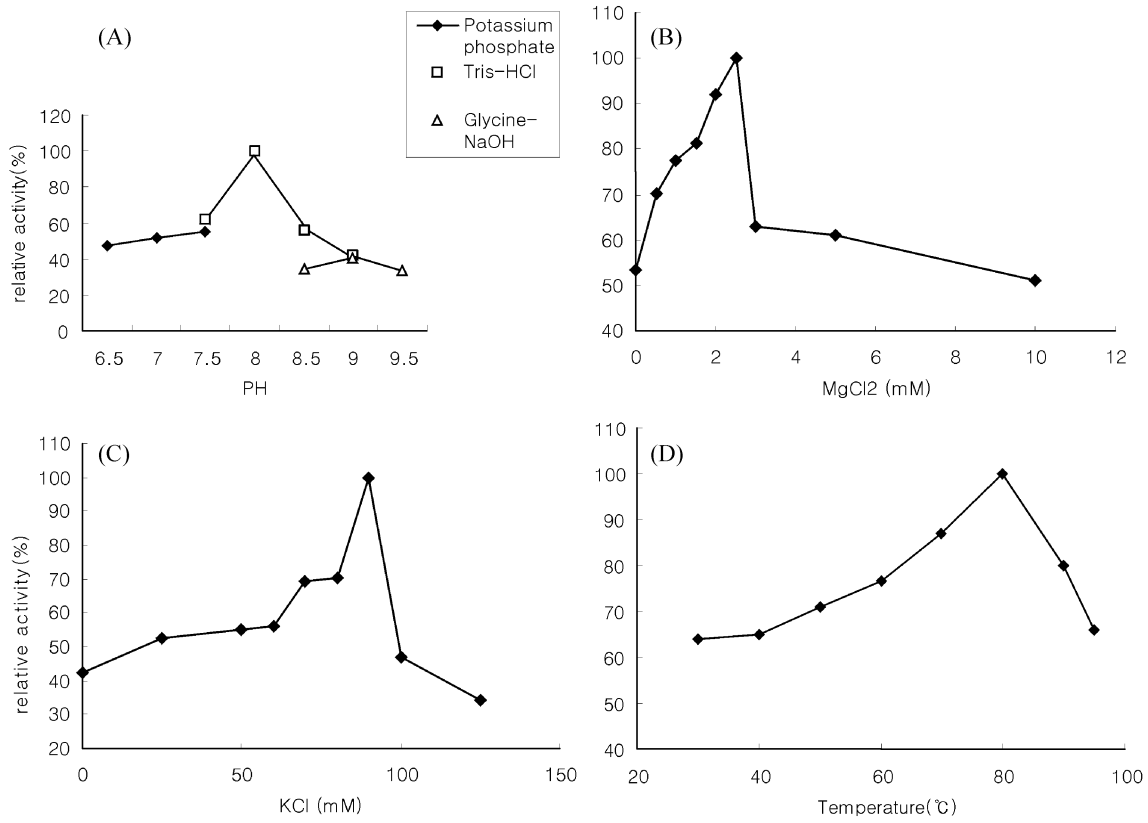

Fig. 3. Effects of pH (A), $MgCl_2$ (B), KCl (C), and temperature (D) on *Tay* activity

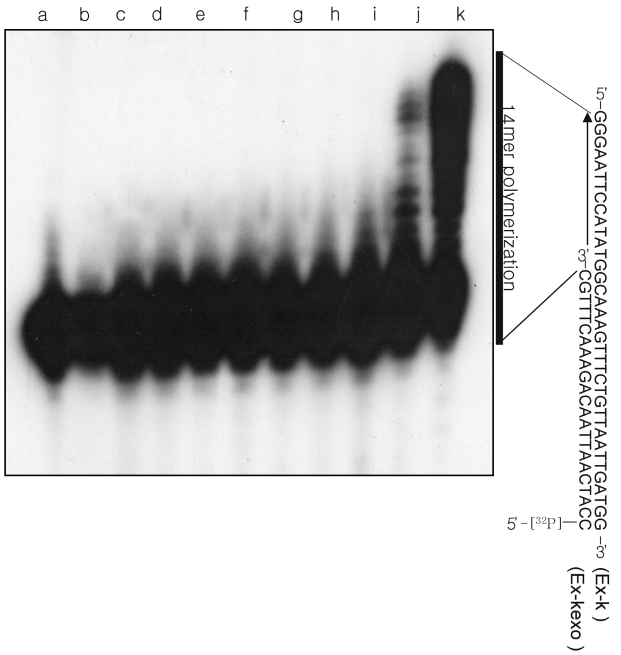

Fig. 4. The nucleotide incorporation dependence on dNTP concentration. Lane (a), primer only; Lane (b), primer assay without dNTP; Lane(c)-(k), assay with primer-template and increasing concentration of dNTP; (c) 0 M, (d) 1 pM, (e) 10 pM, (f) 100 pM, (g) 1 nM, (H) 10 nM, (I) 100 nM, (j) 1 μM, (k) 10 μM.

an assay was performed by monitoring polymerase errors that inactivate the nonessential α-complementation activity of the *lacZ* gene in bacteriophage M13mp18. A gapped M13mp18 substrate was constructed in the single-strand gap that contains the *lacZ* α-complementation target sequence. DNA polymerase was used for the gap-filling synthesis in this assay. If synthesis to fill in the gap in a wild-type DNA substrate is error free, then the α-peptide that is produced from this DNA complements the defective β-galactosidase activity of the host cell to hydrolyze the X-Gal. This results in dark blue M13 plaques. Errors that are introduced during the gap-filling synthesis result in blue or colorless plaques. The fidelity of *Tay* and other reference enzymes are presented in Table 2. The mutation frequency of *Tay* was compared to those of *Taq* and *Pfu* DNA polymerase. *Tay* has higher polymerization fidelity than that of *Taq* DNA polymerase, and lower polymerization fidelity than that of *Pfu* DNA polymerase.

Table 2. Fidelity measurement of three DNA polymerases

Enzymes	Plaques		Mutant frequency
	Total	Mutant	
Taq	718	6	8×10^{-3}
Tay	964	4	4×10^{-3}
Pfu	963	1	1×10^{-3}

In summary, *Tay* DNA polymerase of *T. yonseiensis* was expressed in *E. coli* and purified. *Tay* has a molecular weight of approximately 97 kDa on SDS-PAGE when measured on SDS-PAGE. It does not possess 3'→5' exonuclease activity. The optimum temperature of DNA synthesis is approximately 70-80°C under assay conditions. The optimal magnesium and potassium ion concentrations for DNA synthesis are 2.5 mM and 90 mM, respectively. *Tay* exhibited a half-life of approximately 10 min at 90°C under optimal conditions. This suggests that *Tay* is also a thermostable enzyme. However, the thermostability of *Tay* is not enough for PCR. The PCR and strand displacement amplification (SDA) are two methods of amplifying the nucleic acid sequence. SDA differ from PCR since it is an isothermal amplification process (i.e. all reactions occur at the same temperature without the need for an elevated temperature to melt DNA strands). Primarily, the exonuclease-deficient DNA polymerases have been used in SDA (Walker *et al*, 1992). Therefore, exonuclease activity deficient *Tay* DNA polymerase will be suitably used in SDA with a thermostable restriction enzyme (e.g. *Bso*BI). The *Tay* DNA polymerase will also be used for determining the sequence of single-stranded DNA templates that form stable secondary structures at 37°C. The formation of a secondary structure in the ssDNA template will be prevented, because *Tay* DNA polymerase works efficiently at 70-80°C.

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