Identification and characterization of a helicase-like protein encoded by a *Thermus siphoviridae* phage 4 gene

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Abstract: DNA helicases are essential motor proteins that unwind duplex DNA to yield the transient single-stranded DNA intermediates required for replication, recombination, and repair. As laboratory model strains of thermostable bacteria, the roles of *Thermus* have been studied and discussed extensively. In this study, one gene (ORF42) encoding a helicase-like protein of TSP4 (*Thermus Siphoviridae* phage 4) was identified and characterized. The results showed that ORF42 protein shared a higher homology to the DnaB helicases of *Thermus* bacteriophages P74-26 and P24-46. DNA helicase assay and atomic force microscopy (AFM) revealed that ORF42 protein was an Mg^{2+}-dependent helicase with ATPase activity and involved in DNA unwinding. These evidences indicated that ORF42 protein, homologue of DnaB, probably acts as a helicase in TSP4. This study will not only contribute to explore the co-evolution of *Thermus* phages and their hosts but also shed a new light on the "arm-race" pattern between *Thermus* and its predator (TSP4), providing a basis for the theoretical investigations of new generation bacteriophage therapy.

Keywords: Thermostable bacteria, Bacteriophage, *Thermus siphoviridae* phage 4, DnaB Helicase.

INTRODUCTION

Thermus strains are generally considered as useful models to investigate the mechanism of thermostability of thermophiles, and *Thermus* bacteriophages, typically *Siphoviridae* phages, are known as one of the natural predators to their hosts (*Thermus* species) (Aravind *et al.,* 2004). The *Thermus* have been extensively studied in the laboratory and are irreplaceable in relevant researches, but only several genomes of *Thermus* thermophilus bacteriophages have already been sequenced completely (Minakhin *et al.,* 2008 and Naryshkina *et al.,* 2006). However, sequence analysis of these phages indicated that phages can mostly encode uncharacterized proteins which are dissimilar to those in public databases (Baykov *et al.*, 1981). The co-evolutionary pattern of bacteriophages and their hosts (bacteria) is a unique "arms race" which is far from being elucidated (Stern *et al.*, 2011, Wichman *et al.*, 2005 and Partensky *et al.*, 2011). It is interesting to note that no more than 25% open reading frames (ORFs) of the phage genomes show similarity to the known genes, which indicate the existence of new genes related to the co-evolution of *Thermus* and its phages.

DNA helicases are essential motor proteins that unwind duplex DNA to yield transient single-stranded DNA intermediates required for replication, recombination, and repair (Matson *et al.*, 1990 and Lohman *et al.*, 1996). DnaB helicase is a bacterial enzyme, which opens the replication fork during DNA replication. It is a 5'-3' DNA helicase that may be active in all three stages of DNA replication (initiation, elongation and termination) as well as in recombination and repair. Recently, it has been indicated that the C-terminal region of DnaB is important for DNA binding activity, and its N-terminal region appears to dampen the protein affinity for DNA (Nitharwal *et al.*, 2012). Although bacteriophages can deliver their genomes into their host cells, the underlying molecular mechanisms remain largely unresolved (Bertin *et al.*, 2011). During the infection, the importance of the helicase should not be ignored. Helicase plays central roles in initiation and elongation of DNA replication. For instance, the DnaB protein is the replication fork helicase responding for unwinding dsDNA and attracting DnaB primase during the initiating and elongating actions (Tougu *et al.*, 1994 and Tanner *et al.*, 2008). Consequently, it is vital for chromosome replication in bacteria (Sakamoto *et al.*, 1995 and Smits *et al.*, 2010). DNA helicases couple the hydrolysis of nucleoside triphosphates (NTPs) to the unwinding of duplex DNA (Lahue *et al.*, 1990 and Dou *et al.*, 2014). Coordinating a set of sub-activities (i.e. nucleotide binding, DNA binding and NTP hydrolysis) is required for unwinding of duplex DNA. If the phages have similar helicase genes, which are capable to express in the target bacteria, it will definitely be of disadvantage to the hosts in the arms race.

Due to the fast emerging and widespread of superbugs, for example, pathogenic bacteria that have acquired resistance to almost of all the antibiotics and extreme environment, global efforts have been devoted to developing novel alternative strategies including application of bacteriophages (phages) (Courchesne *et al.*, 2009). Phages from the *Thermus* can be an effectively

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natural resource to inhibit the heat-resistance bacteria. Same mechanisms of DNA replication is applied for bacteriophages, since the *Bacillus subtilis* bacteriophage SPP1 G40P protein (Bárcena *et al.*, 1998), T4 UvsW protein (Carles-Kinch *et al.*, 1997) and T7 Gene 4 helicase (Kong *et al.*, 1998) play a close role as the DnaB of *Escherichia coli*.

As reported previously, we have isolated a novel bacteriophage (TSP4) from a *Thermus* species (Lin *et al.*, 2010). In order to reveal the bacteriophage evolutions, biological features and new functional genes of TSP4, a sequence ORF42 in the genome of TSP4 that potentially encodes a DnaB helicase was used for phylogenetic analysis and functionally characterized. This study will also contribute to explore the interactions between *Thermus* and phages and provide a basis for the application of thermostable DnaB helicase in fields such as molecular biology and pathogen control.

MATERIALS AND METHODS

Sequence analysis of TSP4 ORF42

Putative gene-encoding proteins in the phage genome were found similar to the database protein by BLAST. To analyze the evolutionary relationships, MegAlign software was used for aligning the multiple sequences and drawing the phylogenetic tree. Protein sequences of bacteriophage helicases were obtained from the public databases and were aligned using the MUSCLE software (Edgar 1998). To improve the accuracy of phylogenetic inference, the following factors were excluded: i) non-homologous proteins, ii) sequences were not on the same level and iii) ambiguously aligned regions. The tree topology was inferred by using the appropriate model for DnaB and then was illustrated by TreeView (Page 1996).

Escherichia coli culture

Escherichia coli (*E.coli*) strains DH5α and BL21 (DE3) were used for maintaining plasmids and expressing proteins, respectively. *E.coli* cells were cultured in Luria-Bertani medium (1% Trytone, 0.5% Yeast Extract, 1% NaCl, pH 7.0) at 37°C and supplemented with antibiotic (ampicillin, 100 μg/ml).

Expression and purification of recombinant ORF42 protein

The ORF42 sequence which potentially encodes a DnaB protein in TSP4, was amplified from *Thermus siphoviridae* phage 4 genomic DNA by PCR using the following primers: 5'- CATATGACGGACATAAAGCT GGAG-3' and 5'-CTCGAGGGTAAGAGAGAAATCA AAGTT-3', which was subsequently subcloned into the *Nde*I and *Xho*I restriction sites of vector pET23b (Novagen) after verification by DNA sequencing to generate a recombinant plasmid pET23bORF42, that was further transformed into BL21 (DE3) for expression. When the positive clones were grown to log-phase at 37°C, the 1ml cultures were inoculated into a new 50ml medium in 250ml Erlenmeyer flask followed by addition of 0.5mM IPTG to induce protein expression for 2-3h at 37°C. Bacteria were harvested by centrifugation and lysed with sonication. The supernatant fraction was loaded onto a HisTrap HP column (GE Healthcare) and washed by the buffer A (20mM sodium phosphate, 0.5M NaCl, and 30mM imidazole, pH 7.4), then eluted with a linear imidazole gradient of imidazole (0.01-1M) in buffer A. Protein concentration was determined by Bradford's method (Bradford 1976).

ATPase activity assay of ORF42 protein

The method described by Baykov *et al.*, was used to determine ATPase activity (Baykov *et al.*, 1981). To examine the ATPase activity of ORF42, the purified protein was first tested roughly through absorbance detection using malachite green (Chan *et al.*, 1981). The purified protein ORF42 was incubated in a reaction mixture (20μl) containing 50mM Tris-HCl, 20ng DNA and 25mM $MgCl_2$, at 60°C for 1h. The reaction was stopped by addition of 800μl of 20% $HClO_4$, then 1ml H_2O and 0.5ml dye solution containing malachite green were added respectively. The absorbance was measured at 630 nm by spectrophotometer.

DNA helicase assay

Helicase enzyme activity was determined using the method described by Matson *et al* (Matson *et al.*, 1983). Primer for ORF42 (5'-CAGTGAATTCGAGCTCGGT ACCCGGGGATCCTCTAGAGTCGA-3') was labeled at the 3' end with [γ-^{32}P]-ATP and used as substrate. The complementary strand was added to reach a molar ratio of 1:1.5 between the labeled primer and the unlabeled complementary strand. The reaction mixture was boiled for 3min in reaction buffer (20mM Tris-HCl pH7.5, 100mM NaCl) and cooled to room temperature. The helicase activity assay was done with the mixture containing 50 mM Tris-HCl pH7.5, 10 mM $MgCl_2$, 2mM ATP, 7.5 μg purified ORF42 protein and 10 fmol of [γ-^{32}P]-ATP labeled complementary strand. After incubated at 60°C for 1h, the reaction mixture was placed on ice for 15min and centrifuged at 16,000×g for 15min. 10μl loading buffer (10mM EDTA and 0.1% Bromophenol blue) was added into the terminated mixture and substrate was separated by using 15% polyacrylamide gel. The amount of double-strand DNA and single-strand DNA were quantified via X-ray films.

Atomic force microscopy scanning

The DNA binding and unwinding activities of ORF42 helicase were determined using the method described by Baker *et al* (Baker *et al.*, 1987). The *Nde*I and *Xho*I digested pET-23b (0.5 ng/μl) was incubated together with ORF42 helicase (0.5ng/μl) in the reaction buffer (20mM Tris-HCl pH 7.5, 10mM $MgCl_2$). After 10mM ATP was added to the buffer, the complexes of DNA and protein were deposited onto a fresh mica surface for 10min under room temperature. Then the surface was rinsed slowly with high-purity water and dried with liquid nitrogen. A multimode AFM with a NanoScope IIIa controller (Digital Instruments) was used to take images in air with the 'tapping mode', and the scan rate was 1~2 Hz.

RESULTS

Sequence analysis of TSP4 ORF42 protein

13 representative bacterial and bacteriophage DnaB helicase proteins were selected for phylogenetic analysis. The result showed that, distant from other homologs, close phylogenetic relationship among ORF42 protein, *Thermus* phage P74-26 and P23-45 helicases was observed (fig. 1). Comparison of the deduced amino acid sequence of ORF42 with the DnaB helicase given above by alignment revealed that ORF42 protein contained two conserved motifs, walker A and walker B motif, near the amino acid residues165 and 285, respectively, which are the ATP/GTP and Mg^{2+} binding sites (fig. 2A). ORF42 protein had higher homology with two DnaBs from other *Thermus* phages P74-26 (YP_001468014) and P23-45 (YP_001467899), and ORF42 protein, $DnaB_{P74-26}$ and $DnaB_{P23-45}$ are significantly different from the other DnaBs. Both ORF42 protein and $DnaB_{P74-26}$ consist of 255 amino acid residues with sequence diversity in individual amino acid residues. The predicted structures also coincide with the fact that ORF42 protein is different from other helicase (fig. 2B).

Expression, purification and ATPase activity assay of ORF42 protein

About 46KD ORF42 protein was obtained by pET expression system and purified by HisTrap HP column chromatography (fig. 3). Result of ATPase assay showed that ATPase activity of the purified ORF42 protein was 37±1.1 U/mg. The ATPase activity is defined as the hydrolysis of 1μmol of PO_4^{3-} per minute under the assay conditions. Furthermore, the ATPase activity of the purified protein was not displayed in the reaction buffer without Mg^{2+}, indicating that ORF42 protein was an Mg^{2+} dependent helicase.

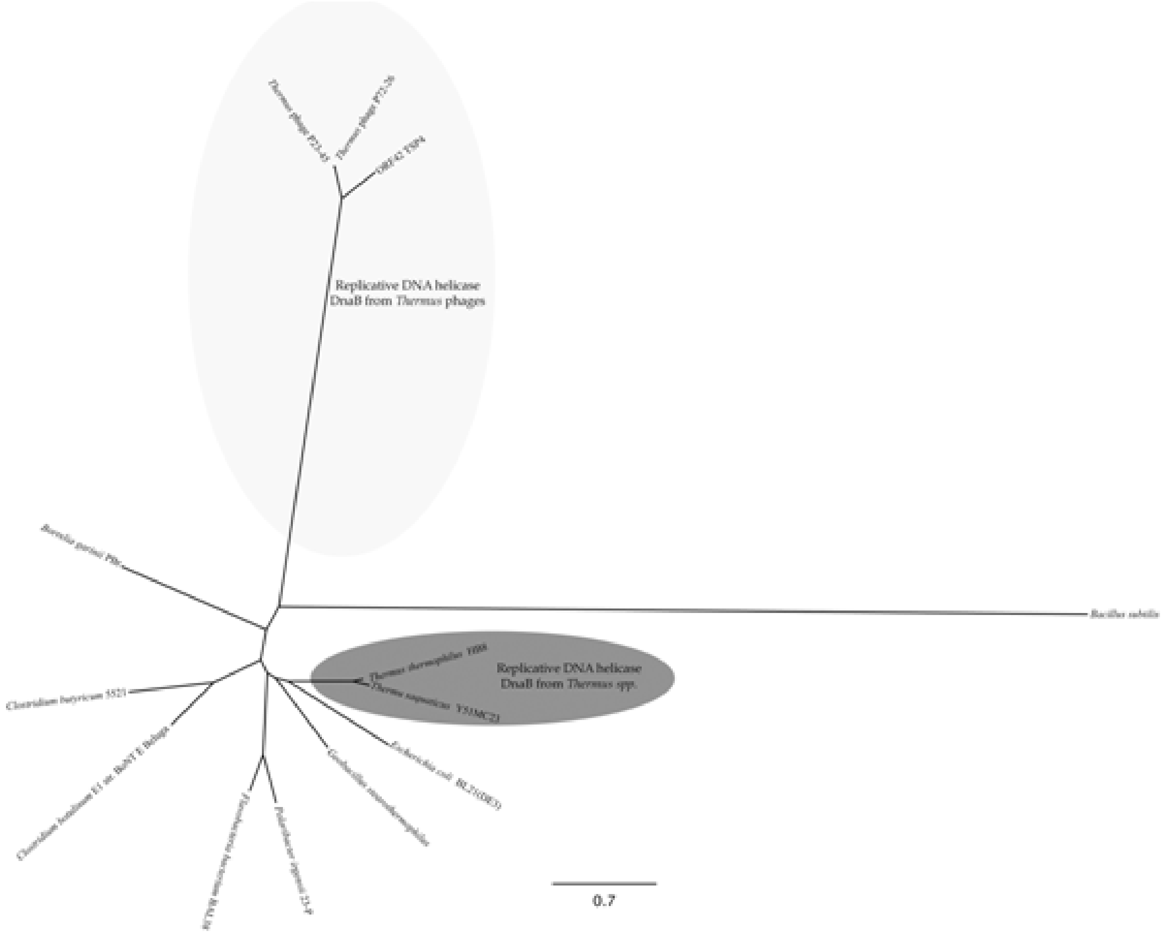

Fig. 1: Phylogeny of 13 DnaB from prokaryotes. The phylogenetic tree was reconstructed using the method of maximum likelihood. Bar, 0.7 substitution per site. The tree is rooted with the nuclear acid sequences of *Bacillus subtilis* DnaB (GenBank accession no. AAC00358). The dendrogram display the evolutionary relationship of DnaB from different prokaryotes. ORF42, DnaB of P74-26 and P24-46 are distant from other homologs.

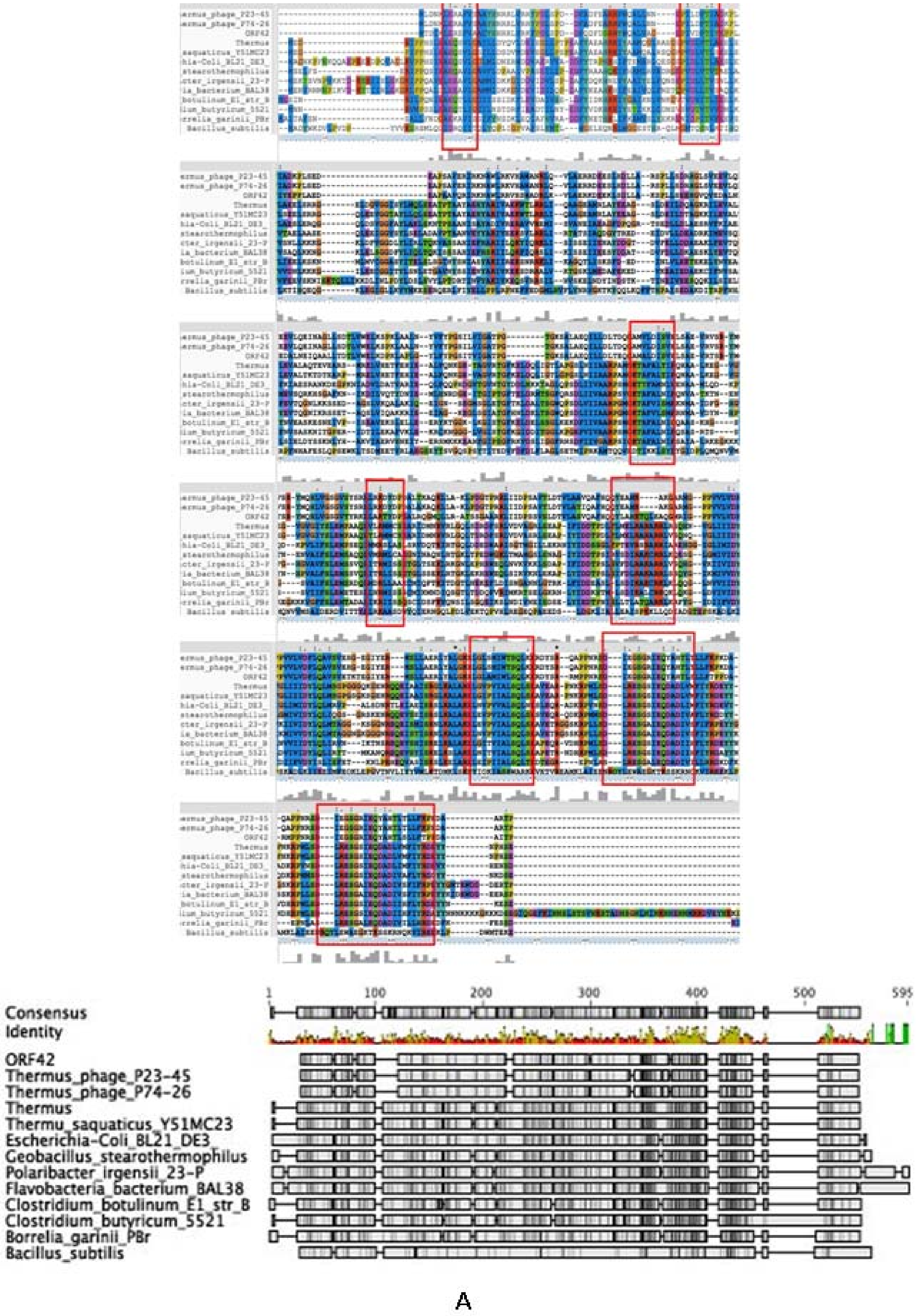

Fig. 2: Three-dimensional structure of ORF42. A, The amino-acid sequence alignment of DnaB were performed with Clustal W2 and the identical residues are indicated by blue black boxes. The conserved regions of DnaB in different prokaryotes are marked in red frames.

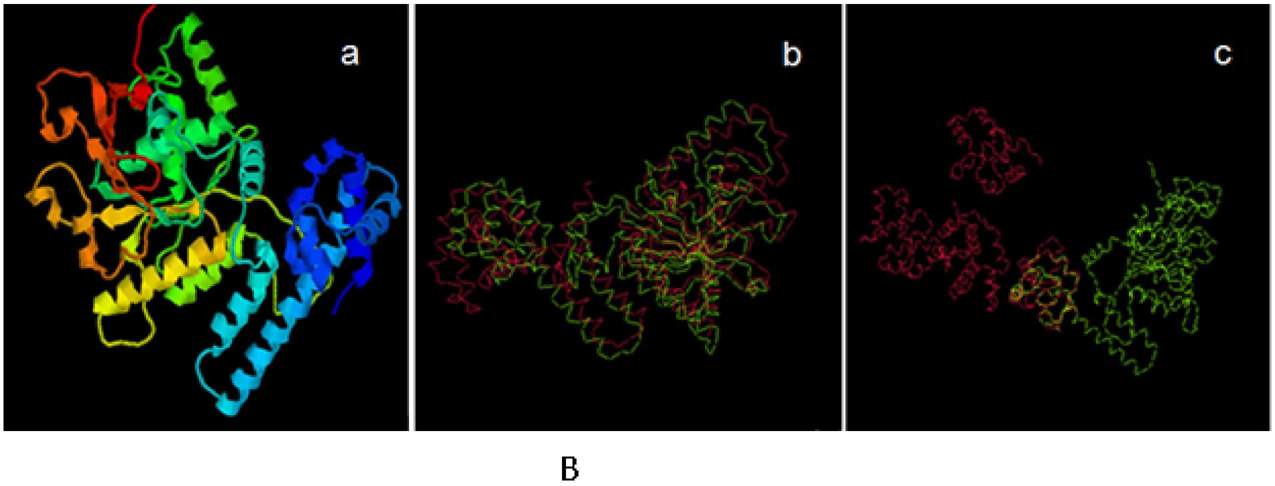

Fig. 2: Three-dimensional structure of ORF42. B, Stereo drawing of the predicted structure of ORF42 (b) and the comparison with the homolog from *E. coil* (showed in red).

Helicase activity of the ORF42 protein

Helicases unwind the duplex DNA to a single-stranded DNA for replication, transcription and recombination. The activity performance of the helicase ORF42 was studied. A 42-nt [γ-^{32}P]-probed primer adopted from the TSP4 genome was cross-linked with the single-strand TSP4 phage genomic DNA. The probed primer dissociated from the single-strand TSP4 phage genomic DNA (the upper band) since ORF42 protein functioned as a helicase. The autoradiography film represented that each lane contained 4.7μg purified protein (fig. 4).

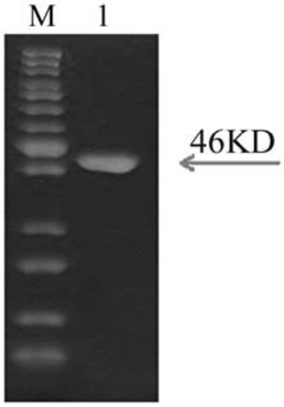

Fig. 3: Expression and purification of ORF42 protein. The molecular weight of the expressed TSP-ORF42 was 46 kD. Lane M, size markers. Lane 1 , purified ORF42 protein.

Unwinding dsDNA by ORF42 Protein

AFM was used to investigate the DNA unwinding activities of ORF42 protein directly and reveal the protein binding to DNA. Replication forks and replication bubble appeared (fig. 5) when ORF42 protein and DNA were incubated together in the reaction buffer.

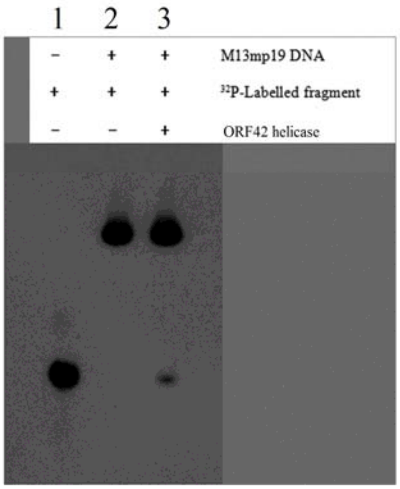

Fig. 4: ORF42 is an ATP-dependent DNA helicase. Lane 1, ^{32}P-Labelled fragment of single-strand DNA for positive control; Lane 2, the annealing fragment without Recombinant helicase for negative control; Lane 3, ORF42 unwind the double-strand DNA into single-strand DNA.

DISCUSSION

Studies have confirmed that bacteria and phages co-evolve in laboratory culture conditions of nutrient medium. Until now, the co-evolution of bacteria and phage mechanism in the natural environment has not been well understood (Gomez *et al.*, 2011). Interactions between bacterial hosts and their phages lead a reciprocal genome evolution through a dynamic co-evolutionary process (Lindell *et al.*, 2007). Previous studies have demonstrated that the DnaB gene encodes a DNA helicase which is essential for unwinding extensive stretches of

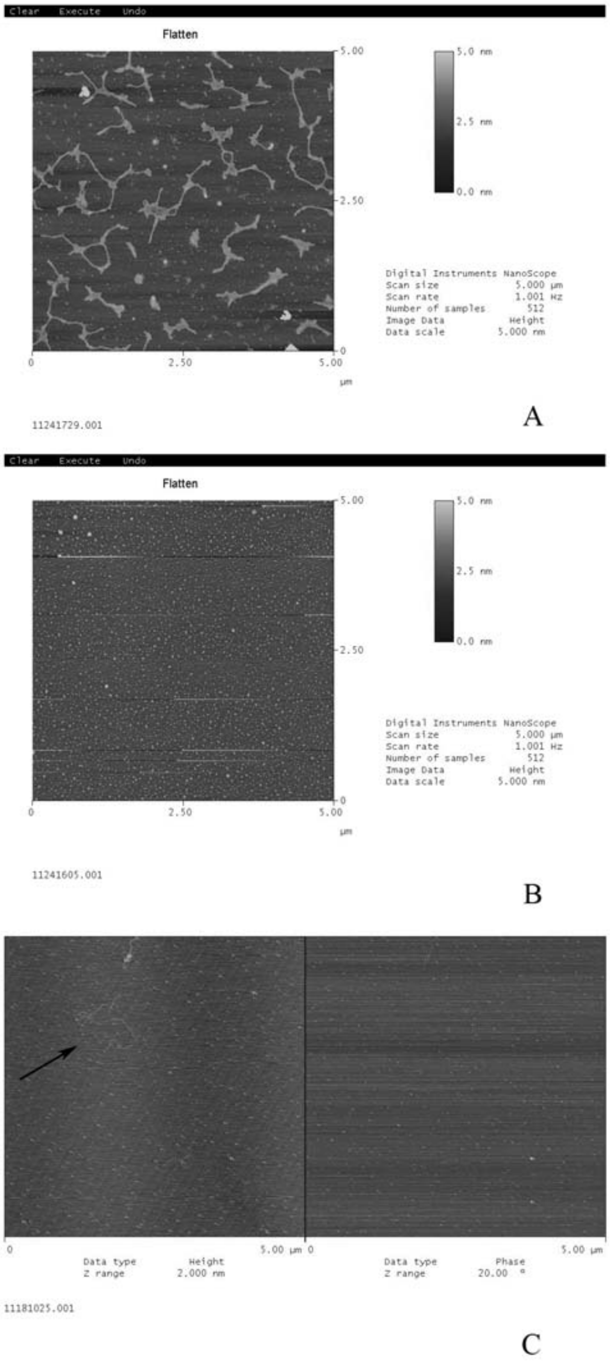

Fig. 5: AFM images of helicase ORF42 and DNA-ORF42 complexes in reaction buffer. (A) ORF42 and the DNA formed a DNA-protein complexes and the DNA was unwinding in the reaction buffer. Adding ORF42 (B) or DNA (C) separately could not unwind the double-stranded DNA.

double-stranded DNA in the presence of ATP (Nitharwal *et al.*, 2007). DnaB were shown to act as a central protein during replication fork moving along the bacterial chromosome. The energy for DnaB activity was provided by NTP hydrolysis. Mechanical energy moves the DnaB into replication fork, and physically splits the fork in half (Eoff *et al.*, 2005). Several DnaB-analog proteins have been isolated from the phages of *E. coil* (Learn *et al.*, 1997 , Günther *et al.*, 1984 , Klein *et al.*, 1980 and Schuser *et al.*, 1979), but none helicase-like protein from *Thermus* phages has been characterized. Regarding the distinct living environment for *E. coil* and *Thermus* spp., the study may provide a new insight into the evolution of bacteriophages.

The present investigation may prove beneficial to the next generation of bacteriophage therapy. As mentioned above, *Thermus* spp. has hardly been eliminated by heat sterilization (Lawyer *et al.*, 1993), which will be a big threat to the public health. Due to the recently erupting antibiotic resistance and virulent bacterial pathogens, bacteriophage therapy has been reconsidered as an efficient "weapon" against bacterial infection (Lu *et al.*, 1993). Despite the current researches and efforts, bacteriophage therapy remains an underutilized option due to challenges such as the host ranges, bacterial resistance, side effects and delivery (Burrowes *et al.*, 2011). Moreover, the infection mechanisms of phages have not been well interpreted, which may not lead to the synthesis of universal and specific phage agents against bacteria. It is still not well known why some bacteriophages encode helicase-like proteins, whereas the others do not. However, helicase-like proteins, obviously, might be helpful for the life cycles of the phages in hosts. Speaking on this point, phages encode helicase-like proteins, like TSP, could be more advantageous in the application of bacteriophage therapy for thermophilic baterica such as *Thermus* spp., in future.

Based on the studies of *Thermus* phage diversity, cloning and expression of the helicase DnaB gene of TSP4 will benefit the understanding of the underlying principles of phage-host co-evolution (Minakhin *et al.*, 2008 and Baykov *et al.*, 1981), the mechanisms of horizontal gene transferred among species and may be applicable to develop unique genetic resources of thermophilic phages as well (Courchesne *et al.*, 2009). In order to prove the homology of two species, further studies can be foucused on alignment of genomic sequences. Consequently, the related proteins in the DNA unwinding process (e.g. DnaG and DnaC) will be investigated, and future research may be able to unveil the complex mechanism of which the phage infect their hosts by using the DNA replication system in the bacteria genomes (Brussow *et al.*, 2011). The full picture of arms race between bacteria and bacteriophages will provide insights into the mysterious co-evolutionary history (Lindell *et al.*, 2007, Stavrinides *et al.*, 2008 and Forde *et al.*, 2008). The present study will not only shed a new light on the "arm-race" pattern between *Thermus* and its predator (TSP4), but also benefit the theoretical investigations of new generation for bacteriophage therapy.

CONCLUSIONS

In this study, the DNA helicase activities of TSP4 ORF42 protein were characterized using the DNA helicase activity assay and atomic force microscopy. The results revealed that ORF42 protein was a Mg^{2+}-dependent helicase with ATPase activity, and ORF42 protein could unwind duplex DNA into single-stranded DNA *in vitro*. These evidences indicated that ORF42 protein, the homologue of DnaB helicase, probably act as the helicase in *Thermus Siphoviridae* Phage 4 *in vivo*.

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REFERENCES

Aravind L, Iyer LM, Leipe DD and Koonin EV (2004). A novel family of P-loop NTPases with an unusual phyletic distribution and transmembrane segments inserted within the NTPase domain. *Genome Biol.*, **5**(5): R30.

Baker TA, Funnell BE and Kornberg A (1987). Helicase action of dnaB protein during replication from the *Escherichia coli* chromosomal origin *in vitro. J. Biol. Chem.*, **262**(14): 6877-6885

Bárcena M, Martín CS, Weise F, Ayora S, Alonso JC and Carazo JM (1998). Polymorphic quaternary organization of the Bacillus subtilis bacteriophage SPP1 replicative helicase (G40 P). *J. Mol. Biol.*, **283**(4): 809-819.

Baykov AA and Avaeva SM (1981). A simple and sensitive apparatus for continuous monitoring of orthophosphate in the presence of acid-labile compounds. *Anal. Biochem.*, **116**(1): 1-4.

Bertin A, de Frutos M and Letellier L (2011). Bacteriophage-host interactions leading to genome internalization. *Curr. Opin. Microbiol.*, **14**(4): 492-496.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248-254.

Brussow H, Canchaya C and Hardt WD (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev.*, **68**(3): 560-602.

Burrowes B, Harper DR, Anderson J, McConville M and Enright MC (2011). Bacteriophage therapy: Potential uses in the control of antibiotic-resistant pathogens. *Expert Rev. Anti Infect. Ther.*, **9**(9): 775-785.

Carles-Kinch K, George JW and Kreuzer KN (1997). Bacteriophage T4 UvsW protein is a helicase involved in recombination, repair and the regulation of DNA replication origins. *EMBO. J.*, **16**(13): 4142-4151.

Chan KM, Delfert D and Junger KD (1986). A direct colorimetric assay for Ca^{2+} -stimulated ATPase activity. *Anal. Biochem.*, **157**(2): 375-380.

Courchesne NM, Parisien A and Lan CQ (2009). Production and application of bacteriophage and bacteriophage-encoded lysins. *Recent Pat. Biotechnol.*, **3**(1): 37-45.

Dou SX, Wang PY, Xu HQ and Xi XG (2004). The DNA binding properties of the *Escherichia coli* RecQ helicase. *J. Biol. Chem.,* **279**(8): 6354-6363.

Edgar RC (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, **5**: 113.

Eoff RL, Spurling TL and Raney KD (2005). Chemically modified DNA substrates implicate the importance of electrostatic interactions for DNA unwinding by Dda helicase. *Biochemistr*., **44**(2): 666-674.

Forde SE, Beardmore RE, Gudelj I, Arkin SS, Thompson JN and Hurst LD (2008). Understanding the limits to generalizability of experimental evolutionary models. *Nature*, **455**(7210): 220-223.

Gómez P and Buckling A (2011). Bacteria-phage antagonistic coevolution in soil. *Science*, **332**(6025): 106-109.

Günther E, Bagdasarian M and Schuster H (1984). Cloning of the dnaB gene of *Escherichia coli*: The dnaB gene of groPB534 and groPB612 and the replication of phage lambda. *Mol. Gen. Genet*., **193**(2): 225-230.

Klein A, Lanka E and Schuster H (1980). Isolation of a complex between the P protein of phage lambda and the dnaB protein of *Escherichia coli. Eur. J. Biochem*., **105**(1): 1-6.

Kong D, Griffith JD and Richardson CC (1997). Gene 4 helicase of bacteriophage T7 mediates strand transfer through pyrimidine dimers, mismatches and nonhomologous regions. *Proc. Natl. Acad. Sci. USA.*, **94**(7): 2987-2992.

Lahue EE and Matson SW (1990). Purified *Escherichia coli* F-factor TraY protein binds oriT. *J. Bacteriol*., **172**(3): 1385-1391.

Lawyer FC, Stoffel S, Saiki RK, Chang SY, Landre PA, Abramson RD and Gelfand DH (1993). High-level expression, purification and enzymatic characterization of full-length *Thermus aquaticus* DNA polymerase and a truncated form deficient in 5' to 3' exonuclease activity. *PCR Methods Appl*., **2**(4): 275-287.

Learn BA, Um SJ, Huang L and McMacken R (1997). Cryptic single-stranded-DNA binding activities of the phage lambda P and *Escherichia coli* DnaC replication initiation proteins facilitate the transfer of *E. coli* DnaB helicase onto DNA. *Proc. Natl. Acad. Sci. USA.,* **94**(4): 1154-1159.

Lin L, Hong W, Ji X, Han J, Huang L and Wei Y (2010). Isolation and characterization of an extremely long tail *Thermus* bacteriophage from Tengchong hot springs in China. *J. Basic Microbiol*., **50**(5): 452-456.

Lindell D, Jaffe JD, Coleman ML, Futschik ME, Axmann IM, Rector T, Kettler G, Sullivan MB, Steen R, Hess WR, Church GM and Chisholm SW (2007). Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. *Nature*, **449**(7158): 83-86.

Lohman TM and Bjornson KP (1996). Mechanisms of helicase-catalyzed DNA unwinding. *Annu. Rev. Biochem*., **65**: 169-214.

Lu TK and Koeris MS (2011). The next generation of bacteriophage therapy. *Curr. Opin. Microbiol.,* **14**(5): 524-531.

Matson SW and Kaiser-Rogers KA (1990). DNA helicases. *Annu. Rev. Biochem.,* **59**: 289-329.

Matson SW, Tabor S and Richardson CC (1983). The gene 4 protein of bacteriophage T7. Characterization of helicase activity. *J. Biol. Chem.,* **258**(22): 14017-14024.

Minakhin L, Goel M, Berdygulova Z, Ramanculov E, Florens L, Glazko G, Karamychev VN, Slesarev AI, Kozyavkin SA, Khromov I, Ackermann HW, Washburn M, Mushegian A and Severinov K (2008). Genome comparison and proteomic characterization of *Thermus thermophilus* bacteriophages P23-45 and P74-26: siphoviruses with triplex-forming sequences and the longest known tails. *J. Mol. Biol*., **378**(2): 468-480.

Naryshkina T, Liu J, Florens L, Swanson SK, Pavlov AR, Pavlova NV, Inman R, Minakhin L, Kozyavkin SA, Washburn M, Mushegian A and Severinov K (2006). *Thermus thermophilus* bacteriophage phiYS40 genome and proteomic characterization of virions. *J. Mol. Biol*., **364**(4): 667-677.

Nitharwal RG, Paul S, Dar A, Choudhury NR, Soni RK, Prusty D, Sinha S, Kashav T, Mukhopadhyay G, Chaudhuri TK, Gourinath S and Dhar SK (2007). The domain structure of *Helicobacter pylori* DnaB helicase: The N-terminal domain can be dispensable for helicase activity whereas the extreme C-terminal region is essential for its function. *Nucleic Acids Res*., **35**(9): 2861-2874.

Nitharwal RG, Verma V, Subbarao N, Dasgupta S, Choudhury NR and Dhar SK (2012). DNA binding activity of Helicobacter pylori DnaB helicase: The role of the N-terminal domain in modulating DNA binding activities. *FEBS J*., **279**(2): 234-250.

Page RD (1996). TreeView: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci*., **12**(4): 357-358.

Partensky F and Garczarek L (2011). Microbiology: Arms race in a drop of seawater. *Nature*, **474**(7353): 582-583.

Sakamoto Y, Nakai S, Moriya S, Yoshikawa H and Ogasawara N (1995). The *Bacillus subtilis* dnaC gene encodes a protein homologous to the DnaB helicase of *Escherichia coli*. *Microbiology*, **141**(Pt 3): 641-644.

Schuster H, Lanka E, Edelbluth C, Geschke B, Mikolajczyk M, Schlicht M and Touati-Schwartz D (1979). A dnaB-analog DNA-replication protein of phage P1. *Cold Spring Harb. Symp. Quant. Biol*., **43(** Pt 1): 551-557.

Smits WK, Goranov AI and Grossman AD (2010). Ordered association of helicase loader proteins with the *Bacillus subtilis* origin of replication *in vivo. Mol. Microbiol*., **75**(2): 452-461.

Stavrinides J, McCann HC and Guttman DS (2008). Host-pathogen interplay and the evolution of bacterial effectors. *Cell Microbiol*., **10**(2): 285-292.

Stern A and Sorek R (2011). The phage-host arms race: shaping the evolution of microbes. *Bioessays*, **33**(1): 43-51.

Tanner NA, Hamdan SM, Jergic S, Loscha KV, Schaeffer PM, Dixon NE and van Oijen AM (2008). Single-molecule studies of fork dynamics in *Escherichia coli* DNA replication. *Nat. Struct. Mol. Biol.*, **15**(2): 170-176.