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Cloning, expression, purification and characterization of replication protein from plasmid pGP2 from *Acetobacter estunensis*

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ABSTRACT

The *Acetobacter estunensis* Rep_{34} protein participates in the replication of bacterial plasmid pGP2. The Rep_{34} protein of the *A. estunensis*, was cloned to the expression vector, that ensure fusion with a His-tag sequence (Rep_{34} His-tagged), over-expressed in *Escherichia coli* and purified by metal-affinity chromatography to yield a highly purified and active protein. On this purified protein number different activities and motifs were detected. DNA band-shift assays showed that the Rep_{34} His-tagged protein bound to the regulation region for replication on the linear double-stranded DNA. In the protein was determined phosphatase activity, ATPase activity and protein is possible to unwind double strand DNA.

Keywords: Acetobacter Estunensis; Rep_{34} Protein; DNA-Binding Activity; ATPase Activity; Phosphatase Activity; Unwinding Activity

The replication of eukaryotic and prokaryotic chromosomes, bacteriophages and bacterial plasmids DNA involves several analogous events and similarities in replisome architecture. Many systems have specific initiation proteins, including bacterial DnaA protein, phage lambda O protein, plasmid replication initiation proteins (Rep) and the eukaryotic origin recognition complex (ORC). The specific mechanism for replication initiation of a given replicon is dependent on both the structure of the replication origin and the nature of the replication initiation protein. The replication of bacterial extra-chromosomal replicons, such as plasmids or phages is generally limited to a single host or a few closely related host´s proteins [1]. Replication proteins generally initiates and regulates bacterial chromosome or plasmid replication and serves as a transcription factor [2,3].

The origins of prokaryotic and some eukaryotic replicons possess characteristic functional elements, including specific binding sites for the appropriate initiation protein and an AT-rich region where DNA duplex destabilization occurs. Plasmid origins usually contain multiple binding sites (iterons) for the plasmid-specific replication initiation protein as well as one or more binding sites for the host replication initiation protein, DnaA (DnaA boxes) [1]. These proteins interact with repeated regulation sequences. They also interact with Rep protein or DnaA protein on regulation boxes, which are located within the *ori* regions or the promoter regions or intergenic regions of many bacterial chromosomes and plasmids [4].

The structural elements of the origin are employed for broad-host-range plasmid replication and maintenance in different host bacteria species. For example, the minimal origin of the broad-host-range plasmid RK2 possesses five iterons and is functional in *E. coli*. However, the presence of three iterons stabilizes RK2 plasmid maintenance in *Pseudomonas putida* [5]. In addition, the region with four DnaA boxes is essential for RK2 replication in *E. coli*, but is dispensable for replication of the plasmid in *Pseudomonas aeruginosa* [6,7].

In the *E. coli* chromosome, the replication origin (*oriC*) contains five DnaA box sequences. The binding of multiple DnaA molecules in the presence of the histone-like HU protein and the site-specific DNA-binding protein IHF (integration host factor) results in destabilization of the duplex DNA within the nearby AT-rich sequences of the *oriC* of *E. coli* [3]. Origin opening of the narrow-host-range plasmids pSC101, F, P1, and R6K requires, in addition to *E. coli* DnaA, HU and/or IHF proteins, the binding of plasmid-encoded replication initiation proteins [8-12]. Similarly, the formation of an open complex at the replication origin of the broad-host-range plasmid RK2 by the plasmid encoded TrfA initiation protein requires *E. coli* HU, and is stabilized by *E. coli* DnaA [13].

In contrast to the chromosomal *oriC*, plasmid origins

do not require ATP for open complex formation [8-10]. A basis for this lack of dependence on ATP, induced in an ATP-independent mode, by the complex of the plasmid-encoded Rep protein and the host HU or IHF [9,14,15].

Plasmids encoded systems that control their replication such that fairly precise, steady-state copy numbers are maintained [16]. Plasmid replicons from Gram-negative bacteria always seem to encode a negative feedback control system. Two basic mechanisms for the regulation of plasmid replication have been recognized so far: one operates via an antisense RNA transcript that negatively regulates the replication; the other operates via iterons, a series of direct repeat sequence located within *ori* that intereract with iteron-binding Rep proteins are responsible for both the initiation of replication and its control [16,17].

From acetic acid bacteria were purified and characterised several DNA plasmids which encoded *rep* gene which product is able to regulate replication process. The first identified cryptic plasmid from *Acetobacter* encoding Rep protein had been used for the construction of cloning vectors [18,19]. Later from *Acetobacter pasteurianus* was identified large plasmid pAC1 [20], and pAP12875 [21]. From *Gluconobacter* was isolated plasmid pJK2-1 [22], and from *A. aceti* plasmid pAG20 [23].

In this paper we presented replication protein of plasmid pGP2 isolated from *Acetobacter estunensis* GP2 strain and characterise main activities that belong to the bacterial replication proteins.

1. MATERIALS AND METHODS

1.1. Bacterial Strains and Cultivation Media

Escherichia coli strain XL1 Blue (tetracycline^R) [24] was used for plasmid isolation and for cloning DNA fragments and strain BL21 (DE3) [F^- *ompT gal dcm lon* $hsdS_B(r_B^- m_B^-)$ λ(DE3 [*lacI lacUV5-T7 gene1 ind1 sam7 nin5*]) was used as a host for protein expression. The pGP2 plasmid isolated from *Acetobacter estunensis* GP2 used as template for rep_{34} gene. *Acetobacter* strain was cultivated in YPG medium (5% yeast extract, 3% peptone, and 1% manitol) and *E. coli* strain on LB medium (10% Tryptone, 5% yeast extract and 5% NaCl pH 7.4) supplemented with 50 µg/ml kanamycin and 100 µg/ml ampicillin.

1.2. Biochemical Material

The reagents for PCR and oligonucleotide primers were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). The bacterial vector pGEM-T Easy (ampicillin^R; Promega, Madison, WI, USA) expression pET-28a+ vector (T7 promoter, kanamycin^R; Novagen, Madison, WI, USA), was used for cloning and expression *rep* gene. Restriction endonucleases, isopropylthio-β-D-galactopyranoside (IPTG), and T4 DNA ligase were obtained from BioLabs. *p*-nitrophenyl phosphate (pNPP) and protein standard used as SDS–PAGE marker were from Sigma Chemical (St. Louis, MO, USA).

1.3. Cloning and Expression Vector Construction

Recombinant DNA techniques were performed using conventional protocols. The rep_{34} gene of *Acetobacter estunensis* (ORF2) was amplified using: forward 5′-GGA TCC ATG TGG TAT CAA AAG ACG CT–3 and reverse primer 5′-AAG CTT TTA TTC AGA TGG CGG CTT G–3′. The amplified DNA encoding the rep_{34} gene produced a fragment of around 627 bp was cloned into pGEM-T Easy vector and transformed in *E. coli* XL1. Selected construct pGEM-rep_{34} was sequenced by dideoxy chain termination method [25] using an ABI Prism 3200 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequenced data were analyzed using BLAST-Basic local alignment search tools program [26] through the network service of the National Center for Biotechnology Information (http://ww.ncbi.nlm.nih.gov).

The DNA encoding the rep_{34} was then sub-cloned into vector pET-28a- in BamHI and HindIII sites which was used to transform *E. coli* XL1. The new vector construct was named pET–rep_{34}. Sequencing of the cloned vectors revealed the open reading frame (ORF2) of the rep_{34} gene plus the expected 32 additional amino acid residues derived from pET-28a- vector at its amino terminus (MGSSHHHHHHSSGLVPRGSH MASMTGGEEMGR), including the cluster of six histidine residues for protein purification by metal affinity chromatography. The expression vector pET-rep_{34} was extracted from the transformants using JETQuick Plasmid kit (Genomed), and used to transform to *E. coli* BL21 (DE3) competent cells. Selected transformant pET-28-rep_{34} was used for protein expression and purification.

1.4. Expression and Purification of Recombinant Rep_{34}

A total of 100 µl of an overnight culture of *E. coli* BL21 (DE3) with pET-28-rep_{34} was diluted into 100 ml of LB medium containing kanamycin (50 µg/ml). The culture was grown at 37℃ until optical density at 590 nm reached 0.6 and was induced with 0.5 mM IPTG. The incubation continued for an additional 2 h in the same temperature. After incubation, the culture was harvested by centrifugation at 8,000 g for 10 min at 4℃. The pellet cells were suspended in 25 mM Tris.HCl, pH 8.0 buffer

containing 20 mM NaCl, and 5% glycerol and disrupted for 10 min at 4℃ followed by sonication. The suspendsion was then centrifuged at 16,000 g for 10 min at 4℃ to separate the cell debris, and the solubility of the recombinant fusion protein was analyzed by 12% SDS–PAGE. The soluble fraction containing recombinant pET-*rep*$_{34}$ was loaded onto a Ni–NTA column (Qiagen, Hilden, Germany) pre-equilibrated with 50 mM sodium phosphate, pH 8.0 buffer containing 300 mM NaCl and 10 mM imidasole. The unbound proteins were washed out with the same buffer used for equilibration of the column. Subsequently, the recombinant proteins were eluted with the same buffer containing 50 to 250 mM imidazole. The resulting pET-*rep*$_{34}$ was exhaustively dialyzed in 50 mM Tris.HCl, pH 8.0 buffer containing 50 mM NaCl for elimination of imidazole. The protein purity was confirmed by the presence of a single band on SDS–PAGE 12% of molecular weight predicted for the Rep$_{34}$ (about 25.5 kDa, with His-tag). For protein visualization, the gels were stained with Coomassie Brilliant Blue G-250 (Bio-Rad Laboratories) and distained with 10% acetic acid and 20% methanol. The purified protein was frozen and stored at –80℃. Protein concentration recombinant soluble protein concentration was determined by UV absorbance at 280 nm (Spectrophotometer ND-1000 UV-Vis, NanoDrop Technologies).

1.5. Computational Methods for Secondary Structure Prediction

The DNA nucleotide sequence was translated into a protein sequence and the deduced amino acid sequence was analyzed using the Expasy SwissProt Web server (http://www.expasy.ch). The sequence of amino acids from Rep$_{34}$ was aligned with 45 sequences of GST enzymes using ClustalW [27]. The methods used for general secondary- structure prediction were Jpred [28], PHD [29], PSIPRED [30], and SSpro [31]. The predicttion of secondary structure and analysion of protein motifs were made using program CLC Main Workbench 5.1.

1.6. Assay of ATPase Activity

The ATPase activity was assayed by using non radioactive modified method [32]. With interaction of free anorganic phosphate with fresh prepared 0.045% (w/v) malachit green and 4.2% (w/v) molibden ammonium in 4 M HCl in the rate of 3:1. Reaction mixture contained in 50 µl (1 µg protein, 50 mM Tris-HCl pH 7.9, 5 mM $MgCl_2$ and 1 mM ATP) at 37℃ in times volume 0, 5, 10, 15, 20 min. After reference times was added 800 µl malachit-molybdene solution and after 1 min added 100 µl of 34% (v/v) sodium citrate and the absorbance change was measured spectrophotometrically at 660 nm. As a standard was used reaction with different concentration of Na_3PO_4.

1.7. Helicase Assay

Helicase activity was detected by the release of a Fam3-labeled oligonucleotide annealed to M13mp18 single-stranded (ss) DNA [33]. The oligonucleotide, which consisted of 20 bases (5′ – fam3-GTT GTA AAA CGA CGG CCA GT – 3′) was complementary to the M13 DNA. Labeled oligonucleotide was annealed with M13 ss DNA (New England BioLabs) in 10 mM Tris.HCl (pH 8.0), 1 mM EDTA, and 100 mM NaCl, heat for 5 min at 65℃ and slowly cooling down for 30 min to room temperature. The double-strand (ds) substrate was purified by ethanol precipitation to remove unannealed oligonucleotide. The helicase reaction mixture contained 5 nM substrate in 20 mM Tris.HCl, 2 mM DTT, 5 mM $MgCl_2$, 5% glycerol, 5 mM ATP, 0.1 mg/ml BSA, and the solution was adjusted to pH 7.5. The reactions were incubated at 37℃ for 30 min and stopped adding 1/10 volume of 3 M sodium acetate and ethanol precipitate. After precipitate pellet was suspended in 100 µl of sterile water and unwound ds DNA substrate was quantified by fluorometric analysis at 492 nm on fluorescence analyzer (Tecan Safire 2 Microplate Fluorescence Reader). DNA unwinding was calculated as a percentage of the total counts in each reaction.

1.8. Gel mobility Shift Assays

The DNA probe for the electrophoretic mobility shift assay (EMSA) was the plasmid pGP2 transcription and replication region in position 1671-2761 bp. The probe was synthesized by PCR, using the primers 5’ – GAG CTC ATG CAT GTA CGC CGC GGT – 3’ and 5’ – AAG CTT TTA TTC AGA TGG CGG CTT G – 3’. After amplification, the DNA probe was cleaved by PvuII which created two fragments 539 and 475 bp. Mobility shifts were performed as previously described [34], with some modifications. The reaction mixtures (15 µl) contained 50 mM Tris.HCl (pH 8.0), 50 mM NaCl, 4.8 mM dithiothreitol, 1.0 mg/ml bovine serum albumin per ml and 20% (w/v) glycerol. DNA probe, reaction mixture and recombinant Rep34 protein (0.4 pmol) were incubated at 30℃ for 15 min. The separation take place on a 4% polyacrylamide gel containing 0.09% bis-acrylamide, 2.5% glycerol, and 1x TBE at 5-10 V/cm. The complexes were visualized after colored ethidium bromide [35].

1.9. Phosphatase Activity

The enzymatic activity of Rep$_{34}$ towards *p*-nitrophenyl phosphate (pNPP) substrate was assayed at 37℃ by spectrophotometric detection of the absorbance at 405 nm due to the release of *p*-nitrophenol (pNP) [36].

Kinetic measurements assigned to determine substrate kinetic parameters were performed in reaction mixtures of a total volume of 400 µl. The mixtures contained 50 mM Tris–acetate, pH 5.5, 50-100 nM Rep_{34}, in the presence or absence of 10 mM $MgCl_2$, and different concentrations of pNPP. The reactions were initiated by the addition of the enzyme, and quenched after 10 min by the addition of 100 µl of 2 M NaOH followed by centrifugation at 14,000*g* for 5 min. The absorbance at 405 nm was read for supernatants of reaction mixtures (containing enzyme) and controls (the same substrate concentration omitting the enzyme), and the difference between these measurements represented the real rate of product formation.

1.10. Protein Analysis

Proteins were analyzed by SDS–PAGE [37] stained either by Coomassie Brilliant Blue R-250 method. Protein concentrations were determined by the Bradford procedure [38] or by densitometric analyses of Coomassie Brilliant Blue-stained SDS–PAGE gels. Bovine serum albumin (BSA) was used as the standard.

2. RESULTS AND DISCUSSION

Bacterial plasmid pGP2 purified from *Acetobacter estunensis* GP2 (2 797 bp) encoded three proteins. ORF2 encoded 209 aa large protein belonging to group of replication protein designed as Rep_{34} (**Figure 1**). By the analysis of amino acid sequence were determined the number of alpha helixes (4 larger than 4 aa) and beta structures (4 larger than 4 aa) and two domains: Helicase conserved C-terminal domain (137-175 aa) and HTH motive (169-203 aa). Isoelectric point of this protein, determined at pI 9.25, is similar to the isoelectric point determined in replication proteins of plasmid pAG20 from *A. aceti* 3620 (pI 8.2) [23], but a bit distinct to isoelectric point of protein from pAP12875 plasmid from *A. pasteurianus* (pI 12.1) [21].

The *rep* gene was amplified using PCR amplification and this product was cloned into pGEM-T easy vector (pGEM-rep_{34} recombinant). Re-cloning gene in pET28a-expression vector was in *E. coli* BL21 (DE3) cells amplified protein under T7 promoter and was constructed pET28-rep_{34}.

2.1. Protein Purification

The expression vector containing the encoding rep_{34} gene was used to transform to *E. coli* BL21 (DE3) cells, which over expressed a Rep_{34} protein after IPTG induction. An over-expressed band on SDS–PAGE corresponding to a protein of approximately 25.55 kDa (with His-tag) was observed in the crude bacterial lysates, consistent with that expected for the recombinant protein (**Figure 2(a)**). The greater part of Rep_{34} was found in the supernatant after lysis and after using affinity chromatography, the recombinant protein was quickly purified to apparent homogeneity (**Figure 2(b)**). More detailed data and purification steps were presented in **Table 1**. The total protein yield at the last purification step was approximately 71 mg of Rep_{34} per liter of bacterial culture.

Upon SDS–PAGE under reducing conditions, the isolated protein migrated as a large homogenous band with a molecular weight between 20 and 30 kDa, consistent with that expected for a Rep_{34} monomer. A monomer of the recombinant protein has a predicted molecular weight of approximately 23.31 kDa. The molecular mass of Rep34 is lower than the molecular mass of RepA monomer protein described in plasmid pRSF1010 that was determined on 31 kDa [39]. The results of the secondary-structure prediction indicate that overall Rep_{34} is composed of the same amount of α-helical and β-sheet conformation.

2.2. In Vitro DNA-Binding Activity

DNA band-shift assays showed that the purified Rep_{34} His-tagged protein was able to bind specifically to linear double-stranded amplified 1091 bp fragment from replication region of plasmid pGP2 in position 1670-2761 bp. PCR product was cleaved by PvuII and afford two fragments 475 bp and 539 bp which were used as substrate for gel shift assay. Incubation of increasing concentrations of Rep_{34} with a fixed amount of DNA progressively altered the mobility of the DNA, indicating the formation of protein–DNA complexes. Since the Rep_{34} apparently has sequence specificity. Furthermore, the increase in DNA band retardation dependent on the Rep_{34} protein concentration is probably due to the progressive occupation of the DNA molecule by Rep_{34} (**Figure 3** lane 2-6).

Interaction Rep_{34} protein with substrate DNA was specific on the 539 bp large fragment encoded regulation region for transcription of replication protein as well as *ori* region of plasmid pGP2. The second smaller DNA fragment 475 bp did not change its position during experiment and showed that it is not a specific substrate for analyzed protein.

2.3. Assay of ATPase Activity

The most of enzymes, that catalyse biomolecular reactions involving binding to the DNA and modificate its structure, need energy from ATP dissociation for their right functioning. ATP molecule can be dissociated by the supporting protein or by the enzyme itself. Rep_{34} protein belongs to the second group and the ATPase ac-

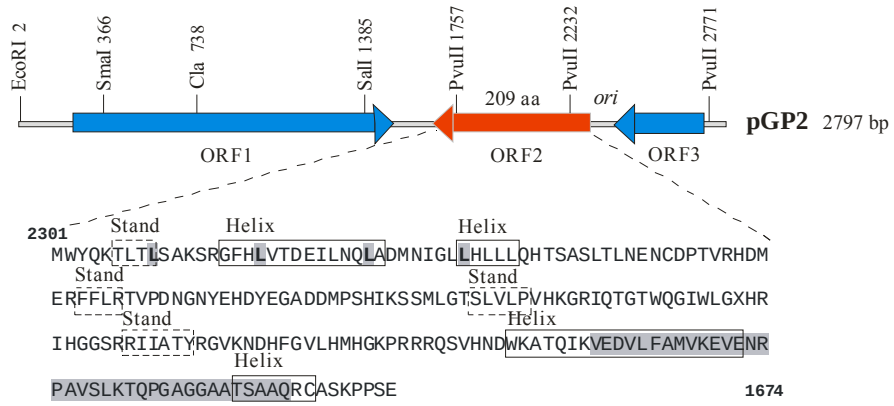


Figure 1. Genetic map of pGP2 plasmid from *Acetobacter estunensis* GP2. Replication protein encoded ORF2 (1674-2301 bp; 209 aa). Gray fraim determined HTH region in protein, full frames determined α helix and dashed frame determined β structure of protein. The gray L represents amino acids residues leucine, that may form the structure of leucine zipper.

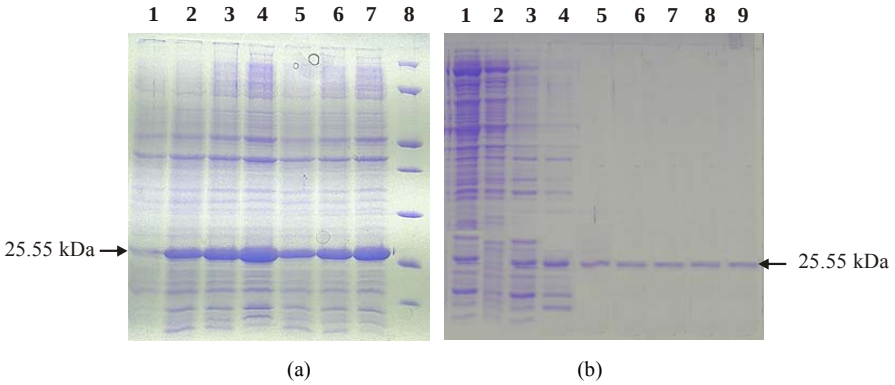


Figure 2. Rep_{34} expression and purification. 12% SDS–PAGE showing: (a) lane 8, molecular weight standard; lane 1, total proteins from cells culture before induction with IPTG; lanes 2-4, total proteins from cells after 0.5, 1, and 2 h of induction with IPTG (0.5 mM IPTG at 37℃), respectively, lanes 5-7, total proteins from cells after 0.5, 1, and 2 h of induction with IPTG (0.5 mM IPTG at 30℃). (b) lane 1, exprimed protein loaded to column, lanes 2, 3 washed column with 5 mM imidazole, lane4-9 recombinant protein Rep_{34} eluted from Ni–NTA resin with 20 mM, 40 mM, 60 mM, 80 mM, 100 mM and 250 mM imidazole, respectively. The Rep_{34} bands are indicated by an arrow approximately 25.55 kDa).

Table 1. Purification of Rep_{34} protein after expression from *Escherichia coli*.

Procedure	Volume (ml)	Protein (mg)	Protein concentration (mg/ml)	Yield (%)	Fold purification
sonification	5	271	54.2	100	1
streptomycin sulphate	4.5	216	48	88	1.25
Ni-affi-Gel	1	7.1	7.1	13	38

Starting material was 0.5 g *E. coli* BL21 (DE3) cell paste

tivity was determined by standard modified method [32] by removing anorganic phosphate from ATP. As showed **Figure 4**, one microgram of protein converse about 0.58 ± 0.11 μg/min ATP to ADP. This activity is higher than the ATPase activity of SecA protein from *E. coli* (0.32 μg/min) [40], but similar to the products of *rep* genes from *E. coli* [41] and bacteriophages replication proteins [42].

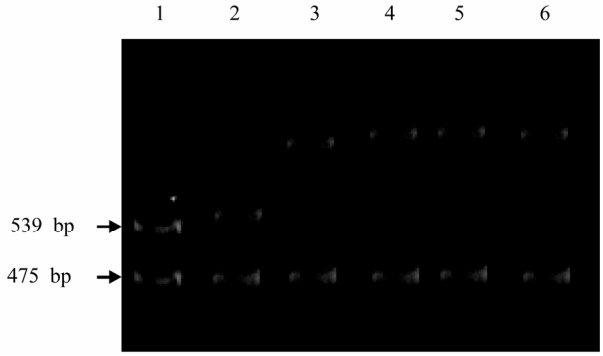


Figure 3. DNA-binding activity of the Rep_{34} His-tagged protein. The purified Rep_{34} His-tagged protein was analyzed for DNA-binding activity in band-shift assays using linear two fragments 475 and 539 bp of regulation region of pGP2 plasmid Samples were analyzed in 4% non denaturated PAGE in TBE buffer and visualized after ethidium bromide staining. The bands observed in lane 1 in the absence of added protein and lane 2-6 in presence of different concentration of binding protein (0.1 µg, 0.5 µg, 1 µg, 2 µg, and 5 µg of Rep_{34} protein).

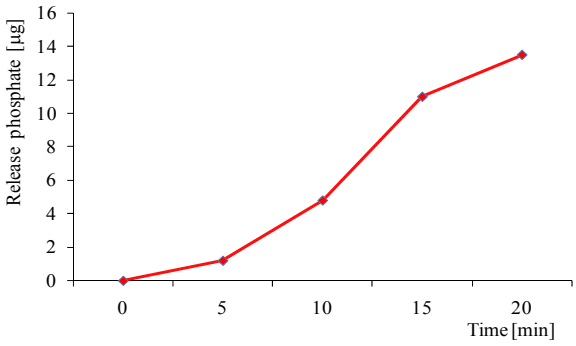


Figure 4. Determination of ATPase activity replication protein Rep_{34} from plasmid pGP2.

2.4. Determination of Phosphatase Activity

Catalytic activity of this protein was studied as an acidic phosphatase, since the highest activity was observed at pH 5.5. Thus, we analyzed the enzymatic activity of Rep_{34} protein and determined the catalytic constants of

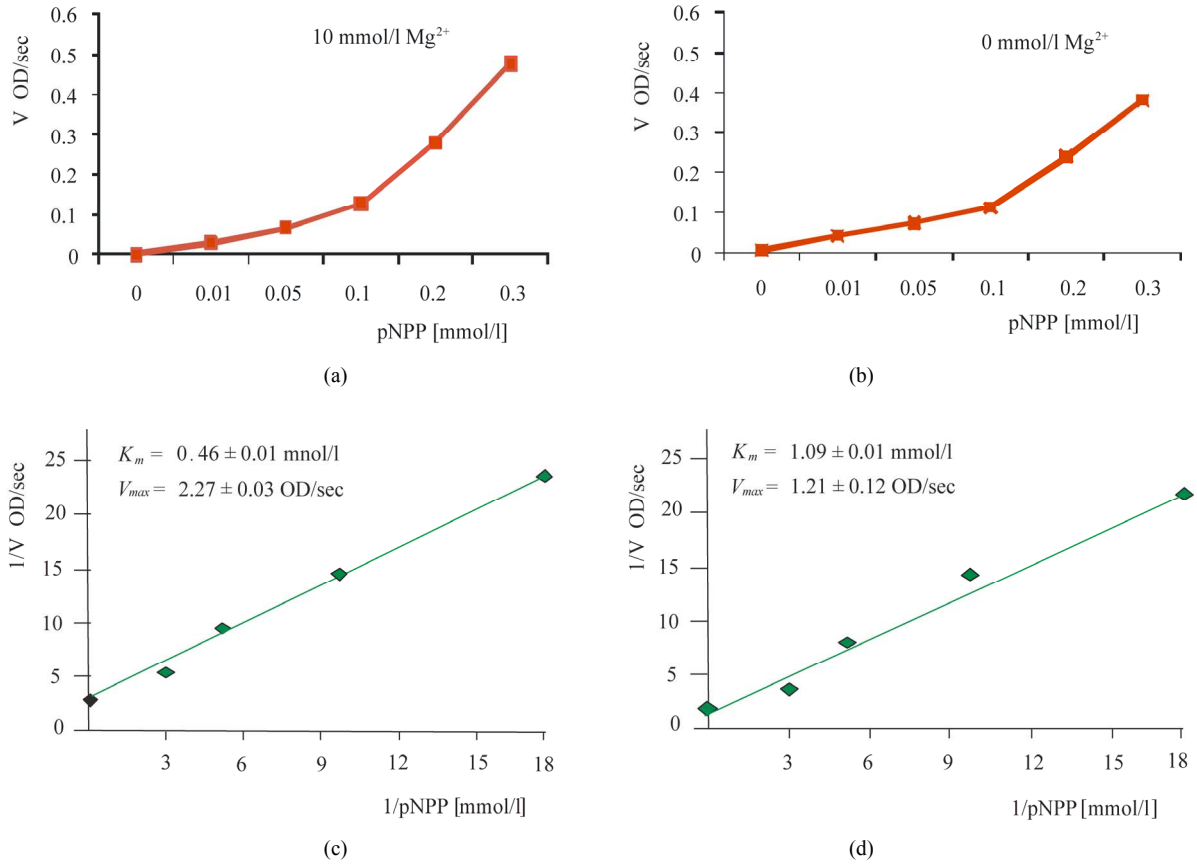
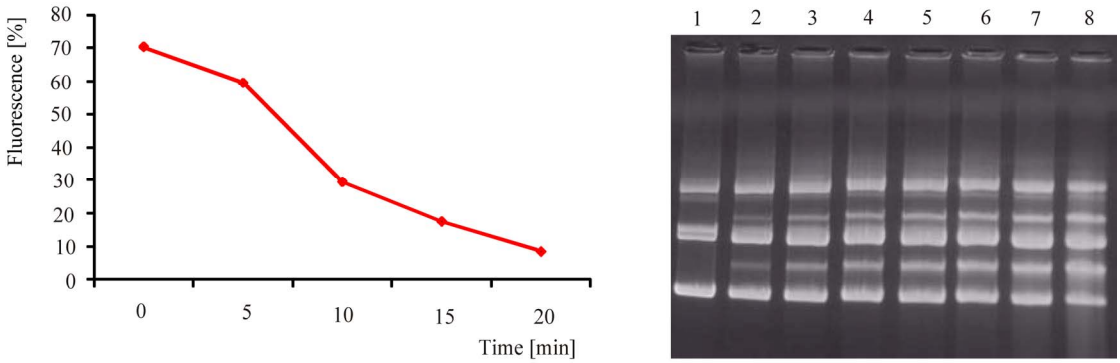


Figure 5. Enzymatic phosphatase activity Rep_{34} protein in the presence or absence of 10 mM Mg^{2+}. (a) Standard Michaelis–Menten curve of Rep_{34} activity assayed with pNPP in the concentration range of 0.01-0.3 mM in the buffer comprising 50 mM Tris–acetate, pH 5.5, 10 mM $MgCl_2$. (b) Standard Michaelis–Menten curve of Rep_{34} activity assayed with pNPP in the concentration range of 0.01-0.3 mM in the buffer comprising 50 mM Tris–acetate, pH 5.5. (c) Lineweaver–Burk plot of the kinetic process presented in (a) was used to derive K_m and V_{max}. (d) Lineweaver–Burk plot of the kinetic process presented in (b) was used to derive K_m and V_{max}.



(a) (b)

Figure 6. A representative DNA helicase assay in the presence of ATP. (a) Effect of decrease fam-labeled ds DNA substrate after inteaction with Rep34 protein associed helicase activity. Helicase reactions were incubated at 37℃ for 30 min and the substrate and product were quantified by fluorometric analysis. (b) Unwinding super coiled form of plasmid pGP2 by Rep_{34} helicase activity. Lane 1 standard plasmid pGP2, lane 2-8 activity measured after every 10 minutes of incubation with rep34 protein in optimal reaction condition (OC – open circular, SC – super coiled form).

the protein, using the cleavage of synthetic substrate p-nitrophenyl phosphate. The Rep_{34} was able to cleave pNPP in the presence and in the absence of Mg^{2+} ions (**Figure 5**). Enzyme activity is higher about 20% in the presence than absence Mg^{2+} ions. However phosphatase activity of this protein is about hundred times lower than is described in bacterial acid phosphatases [43]. Assuming a Michaelis–Menten model of the enzymatic activity, we used Lineweaver–Burk plots to derive catalytic parameters K_m and V_{max}. The K_m value calculated in the presence of Mg^{2+} for Rep_{34} is 0.46 ± 0.01 mM and in the absence of Mg^{2+} is 1.09 ± 0.01. The phospthatase activeity is lower than described in bacterial C acid phosphatase of *Helicobacter pylori* (1.20 ± 0.25 mM) [44].

2.5. Helicase Assay

To demonstrate DNA helicase activity, unwinding was monitored by the release of a Fam-labeled 32-mer from a partially double-stranded circular M13 DNA substrate. The release labeled primer from double strand DNA was separated by ethanol precipitation. The pellet was dissolved in water and used for quantification on a fluorescent reader (**Figure 6(a)**). Unwinding activity Rep_{34} protein showed continuously decrease labeled dsDNA after 30 min incubation at 37℃ with 0.5 μg of protein (**Figure 6(b)**). Although a concentration-dependent increase in helicase activity was observed at lower protein concentrations, the amount of unwinding was not significantly increased at greater than 0.5 μg of protein (data not shown).

Finally, new replication protein from plasmid isolated from *Acetobacter* strain was cloned and exprimed in *E. coli* expression systems. Small protein with basic isoelectric point has two domains one for interaction with other replication protein with leucine zipper and second HTH domain for interaction with DNA. Purified replication protein has phosphatase activity, ATP-ase activity and is able to unwind double strand DNA molecule. HTH domain specific recognise boundig region for iniciation replication and transcription of plasmid pGP2. Fusion of this replication protein with GFP protein was used to monitor protein expression by fluorometric microscopy.

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