Chapter 4

Overexpression and purification of Gp07-a lethal protein

4.1. **Introduction**

Temperate bacteriophages are those which can adopt two modes of development-the lytic mode and the lysogenic mode. Some temperate bacteriophages can switch between these two developmental pathways. The switching is induced by certain conditions such as exposure of the lysogen to UV or other agents which can damage the DNA. Under such conditions, the prophage will switch to the lytic mode of development, thereby abandoning the damaged host cell - a process known as prophage induction. In temperate bacteriophages, particularly Phi11 developmental pathway is controlled by two essential repressors primarily, CI and Cro (Das et al 2007; Das et al 2009). However, a handful of temperate bacteriophages such as Coliphage 186 (Shearwin et al 1998), P4 (Liu et al 1998), P1 (Riedel et al 1993), Gifsy1 and Gifsy3 (Lemire et al 2011), have been reported to harbor an antirepressor gene whose product is involved in the lysogenic to lytic switch.

Preliminary report showed that Gp07 upon overexpression led to considerable inhibition of the growth of the host *E. coli* cells; in fact, cell morphology studies confirmed the filamentous morphology of the Gp07 overexpressing cells (Chapter 3: Section 3.3.4.). Though Gp07 has a cell division inhibiting role, hence main objective was to assess its role in the developmental pathway of Phi11.

Despite the importance of antirepressor proteins in the developmental pathway of temperate phages, the aureophage Antirepessor proteins of aureophages have not yet been characterized. The novel architecture of Phi11 antirepressor (amino-terminal Bro and carboxy terminal KilA domain), as well as its inhibitory effect upon host cell division motivated us to carry out the cloning, overexpression and purification of the Phi11 Gp07. The preliminary role of Gp07 showed toxic effect on the expression host; the carboxy terminal domain alone also shows similar activity (Chapter 3). Here we describe a method to optimize the Gp07 expression conditions. This chapter work represents the first step towards the biochemical characterization of antirepressor from *Staphylococcal* phage Phi11 and indicates a possible protocol for efficient overexpression and purification of the protein in a functional, soluble form. This modified protocol might be useful in purification of other similar proteins carrying the KilA and Bro domains.

4.2. **Materials and methods**

4.2.1. **Strains and plasmids**

Phage Phi11, *S. aureus* RN4220, *E. coli* BL21 (λDE3) and *E. coli* XL1 Blue were grown in laboratory conditions as described in section 3.2.1. Appropriate antibiotics (ampicillin and kanamycin) were added to the growth media as required.

4.2.2. **Cloning of *gp07* into expression vector pET28a**

The genomic DNA of Phi11 was isolated as described in section 3.2.4. and used as template for amplification of *gp07* gene. Briefly, to clone Phi11 *gp07*, we employed a polymerase chain reaction with Taq polymerase (DreamTaq DNA polymerase from ThermoFisher Scientific) using primers C-*gp07*-F and C-*gp07*-R (Chapter 3: Table 3.1.). The product obtained was 825bp in size and was cloned into pGEM-T Easy vector according to the manufacturer's protocol (www.promega.com/protocols/) and denoted as pGp07-T. pGp07-T carrying *gp07* was double digested by the restriction enzymes, NcoI and XhoI and sub cloned into expression vector pET28a. pET28a and *gp07* gene cloning sites were generated by double digestion of pGp07-T and pET28a at two sites by the restriction enzyme NcoI and XhoI (restriction sites has been already added to the primer sequences at the time of primer design). PCR products and double digestion fragments were analyzed by electrophoresis on a 1.5% agarose gel and purified using the GeneJET Gel Extraction Kit (Thermo Scientific). pET28a carrying *gp07* has been designated as pGp07 and is derived from the pET28a vector. It has a Lac-regulated T7 promoter and kanamycin resistance. Gp07 cloned in this vector is fused to a carboxy-terminal tag of 8 residues (LEHHHHHH) with 2 amino-terminal residues (MG) to keep the *gp07* gene in-frame. Clones were screened by plasmid purification and followed by PCR and double digestion of recombinant pGp07-T and pGp07. The selected constructs were sequenced at the UDSC, Delhi to rule out any mutation.

4.2.3. **Test expression with time scan for the Gp07 purification**

Vector pGp07 carrying *gp07* gene was transformed into *E. coli* BL21(λDE3) cells to generate expression clones. Small scale test expressions were carried out in 50ml cultures overexpressing the expression clones. Briefly, overnight cultures of the expression clone was prepared in 10ml of Luria bertani broth (LB) medium containing antibiotic (50 µg/ml kanamycin) in a 100ml conical flask, and the culture was grown overnight in a shaking incubator at 37°C, 120rpm. Three sets of 50ml of Luria Bertani broth containing kanamycin (50µg/ml) were inoculated with 1% overnight grown culture and incubated at 37°C with shaking at 120rpm, until an optical density of ~0.4-0.5 was reached at 600 nm (OD_{600}). Expression was then induced by adding 500µM IPTG and cultures

were incubated for three different time points (1hr, 2hr and 3hr) at 32°C with shaking at 140rpm. Cells were harvested separately by centrifugation (6,500rpm for 10 min at 4ºC) and resuspended in 10ml of lysis buffer [20 mM Tris-chloride buffer (pH 7.5), 500 mM NaCl, 5% glycerol, 10 mM imidazole] containing 10 µg/ml of PMSF (phenylmethane sulfonylfluoride) protease inhibitor EDTA-free. This suspension sonicated for 20 seconds on, 2min off cycle (incubated in ice) and five repetitive cycles were used to complete the sonication. Finally, the lysate was clarified by centrifugation (10,000rpm for 20 min, 4ºC) and the supernatant and pellet fraction were separated and the recombinant protein expression was evaluated at the three desired time points. The supernatant and pellet fractions were compared with the un-induced cells by analysis through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS 12%-PAGE). Gels were stained and destained as described in section 2.2.4 (Chapter 2).

4.2.4. **Large-scale protein expression and purification of His-tagged Gp07**

Overnight primary culture of *E. coli* BL21 (λDE3) carrying pGp07 was grown in LB medium containing kanamycin (50µg/ml) in an incubator shaker at 37° C, 120 rpm. 30 ml of the starter culture was used to inoculate 3 liters of LB medium containing kanamycin (50µg/ml) in ten 1 liter conical flasks. Cells were allowed to grow at 120rpm, and 37°C until an OD_{600} ~0.4-0.5 was reached. The culture was then transferred to a shaking incubator at 37°C and shaken at 120 rpm. After a 20 min cool-down period, IPTG was added to a final concentration of 0.5 mM. Cells were further cultured for 1 h at 32° C and 140 rpm. Cells were harvested by centrifugation (10 min, 6,500 rpm at 4°C). The cell pellet was washed with 0.9% NaCl and cell pellet was stored at -80° C until use.

Modified steps for two step IMAC purification has shown positive effect on purification of Gp07. Initially the cell pellet was resuspended in 10 ml of ice-cold lysis Buffer (Lysis buffer: 20 mM Tris-chloride buffer pH 7.5, 500 mM NaCl, 5% glycerol, 10 mM imidazole and 10 µg/ml of PMSF). Cells were lysed by sonication (Misonix Microson Sonifier Misonix XL2000 Sonicator System Ultrasonic Cell Disrupter) on ice at 10 watts (RMS) for 12 min (Repeated cycles of 20 seconds with 2min intervals). Finally, the sonicated extract was centrifuged (45 min, 12,000rpm, 4°C) and the supernatant (Cytoplasmic fraction) was separated from the pellet fraction. Gp07 was purified from the supernantant by Ni-NTA column chromatography according to manufacturer's protocol (ThermoFisher Scientific) using Wash buffer (Wash buffer: 20mM Tris pH 7.5, 500mM NaCl, 25mM Imidazole and 5% glycerol). Finally, the His-tagged Gp07 was eluted from the column using elution buffer (Elution buffer: 20mM Tris pH 7.5, 500mM NaCl, 300mM Imidazole and 5%

glycerol). The elution fractions were pooled together and kept for dialysis against dialysis buffer (Dialysis buffer: 20mM Tris pH 7.5, 500mM NaCl, 0mM Imidazole, 5% glycerol and 1mM EDTA) overnight to allow complete removal of imidazole and EDTA.

The dialysed protein thus obtained was further loaded onto the IMAC column for the next stage of purification. In this case, a different gradient of salt and imidazole concentrations (Wash buffer 1: 750mM NaCl and 25mM Imidazole, Wash buffer 2: 750mM NaCl and 30mM Imidazole, Wash buffer 3: 750mM NaCl and 40mM Imidazole, Wash buffer 4: 500mM NaCl and 60mM Imidazole) with the components of the wash buffer (Tris 20mM pH 7.5, Imidazole, NaCl, 5% glycerol) was incorporated. Finally, Gp07 was eluted using elution buffer (Tris 20mM pH 7.5, 300mM Imidazole, 500mM NaCl and 5% glycerol) and dialysed again for further characterization.

4.2.5. **Western blot analysis**

Western Blotting was performed according to manufacturer's protocol (GE Healthcare, Life Sciences), to confirm the identity of the purified protein. Briefly, the cells expressing the putative C-teminal His-tagged Gp07 were harvested. The putative Gp07 was purified by Ni-NTA affinity chromatography as discussed in section 4.2.4. The elution fraction carrying the purified putative Gp07 protein was run on a SDS-12% PAGE. The resolved proteins were transferred to Immobilon-PSQ Hydrophobic Polyvinylidene Fluoride (PVDF) (Millipore India Pvt. Ltd.) using a sandwich blotter (GE, miniVE System-Blot Module). Transfer was allowed to proceed for 1hr at 4°C at 50 Volts. Following transfer, the membrane was washed with Tris Buffered Saline (TBS) containing 50 mM Tris and 150 mM NaCl, pH 7.6 and blocked with 3% (w/v) BSA for an hour. The blocked membrane was again washed with TBST (50 mM Tris, 150 mM NaCl, 0.1% Tween 20; pH 7.6) and twice with TBS, and finally incubated overnight, with TBS containing primary anti-His antibody (diluted 1:2000) at 4ºC. Next day, the blot was again washed twice with both TBST and TBS for 20 min each and incubated with alkaline phosphatase-tagged goat anti-mouse antibody (IgG1-AP) (diluted 1:2000) for 2h. This was again followed by washing twice with both TBST and TBS for 20 min each and the blot was stained with NBT/BCIP (given in appendix A) (Calbiochem®, Merck Millipore India Pvt. Ltd.) until the signal is clearly visible. The membrane was finally dried and photographed.

4.2.6. **Analytical gel filtration chromatography**

In order to determine the oligomeric status of the purified Gp07 in solution, analytical gel filtration chromatography using a SEC-s2000 column (Phenomenex, USA) was carried out. Briefly, 5 µM of

purified Gp07 was loaded onto a SEC-s2000 column that was equilibrated in dialysis buffer. The protein was eluted at a constant flow rate of 0.5ml/min. The absorbance of the eluted Gp07 was monitored at 280 nm. Marker proteins mix (Aqueous SEC 1, Phenomenex, USA) was separately eluted using same column and conditions. The retention factor of each protein was determined using its retention time, the total volume of the column, and the elution time. The plot of retention factors of marker proteins versus their log of molecular mass was used to calculate the molecular mass of Gp07.

4.2.6. **Glutaraldehyde cross-linking of Gp07**

To determine the protein-protein crosslinking reaction of Gp07 (5µM), the glutaraldehyde-mediated cross-linking experiment has been performed by standard procedure (Das et al 2009). Briefly, 5µM Gp07 (in 0.1M Na-phosphate (pH-7.4), 500mM NaCl and 5% glycerol) was incubated for 20 minutes at room temperature and then treated with 0.1% glutaraldehyde solution for 2 minutes in 20 µl total reaction volume. The reaction was stopped by addition of a SDS sample buffer. The reaction mixtures were boiled for 2 minutes and analysed by an SDS-12% PAGE.

4.2.7. **Spectroscopic studies on Gp07**

The effect of temperature on the secondary structural elements of Gp07 was investigated from the far-UV CD (200-250 nm) spectra of the protein recorded on a Jasco-715 spectropolarimeter. A cell with a path length of 1 mm was used and 5µM of Gp07 was incubated for 5min at different temperatures (15°C, 25°C, 35°C, 45°C, 55°C, 65°C, 75°C and 85°C) (Creighton 1997). The spectra of the protein so obtained were analyzed by BeStSel tool (Micsonai et al 2018). The buffer values were subtracted from the corresponding spectra of protein samples. The scans were repeated thrice for each temperature. PSI-PRED was used to analyse the putative secondary conformation of Gp07 (Buchan et al 2013).

To investigate the effects of GdnCl on the tryptophan (Trp) residues of Gp07, the fluorescence of the samples was measured at room temperature in a disposable 10 mm path length polystyrene cuvette. The intrinsic tryptophan fluorescence spectra (λ_{ex}= 295 nm and λ_{em}= 300-400 nm) of Gp07 (2.5 µM each) were recorded in the presence of GdnCl (using 0M, 1M, 2M, 3M, 4M, 5M and 6M) using a Jasco-FP8500 spectrofluorimeter by the method of Albrecht et al (Albrecht 2008). All resultant spectra were obtained by the subtraction of the control. Three repeat scans per sample were made and the λ_{max} were analyzed.

4.3. **Results**

4.3.1. **Cloning of *gp07* into pET28a expression vector**

gp07 gene was PCR amplified as described above (Materials and methods) and a DNA band of 825bp was observed on a 1.5% agarose gel. This band was gel purified and the product was ligated into a pGEM-T easy vector and transformed into XL1 Blue competent *E. coli* cells. The transformants that had grown on the LB agar plates supplemented with ampicillin (100µg/ml) were subjected to blue white screening and 10 white colonies were selected for further analysis. The 10 colonies were further screened and selected for the presence of *gp07* gene by PCR using forward primer and reverse primer of *gp07*; the DNA isolated from the 10 selected colonies were further subjected to double digestion with NdeI and XhoI. All the ten transformants were found to be positive for the presence of *gp07* and gave a band at 825bp (Figure 4.1.A and 4.1.B) when run on a 1.5% agarose gel.

The sequences of the oligonucleotide primers utilized to generate the pGp07 have already been described (Chapter 3: Table 3.1. and Table 3.2.). The recombinant DNA from a healthy transformant was sequenced to rule out any mutation. Further, a healthy E. coli XL1 Blue single colony harbouring pGp07-T (containing no mutation) was digested with NcoI and XhoI and the *gp07* gene was subcloned into pET28a vector also digested with NcoI and XhoI. Eight transformants were obtained out of which five harboured the recombinant plasmid pGp07 (Figure 4.1.C and 4.1.D). Plasmid DNA was isolated from all the five positive transformants and were individually transformed into *E. coli* BL21 (λDE3) for optimizing the conditions for the overexpression of Gp07.

4.3.2. **Test expression with time scan for the Gp07 purification**

All 5 positive constructs pGp07 were employed for evaluating the optimal conditions for over expression of Gp07 protein production by small-scale test expression (from 25 ml cultures) (Figure 4.2.A). Of the 5 selected positive clones, only construct-I (Cn-I) and to some extent, Cn-II showed optimal recombinant protein expression. However, the growth of *E. coli* BL21(λDE3) transformed with Cn-I was severely impaired, especially after the addition of the inducing agent (IPTG). For the remaining constructs (Cn-IV and Cn-VIII), protein expression levels were found to be very low, while Cn-V, failed to produce any amounts of recombinant Gp07 (Figure 4.2.A).

Test expression results indicated that Gp07 could be produced in a soluble form in optimal quantities in the host cells (Figure 4.2.B). It was further found that high levels of soluble Gp07

protein is produced during 1h of induction with IPTG at 32ºC. When the time of induction was increased to 2h and 3h, soluble protein expression was highly impaired (Figure 4.2.B). Fortunately, a large percentage of Gp07 appeared in the supernatant fraction obtained after sonication, when induced for 1h. Taken together, these results from cloning and small-scale test expression identified the best combination of time and temperature of induction for the production of the Gp07 in a soluble form.

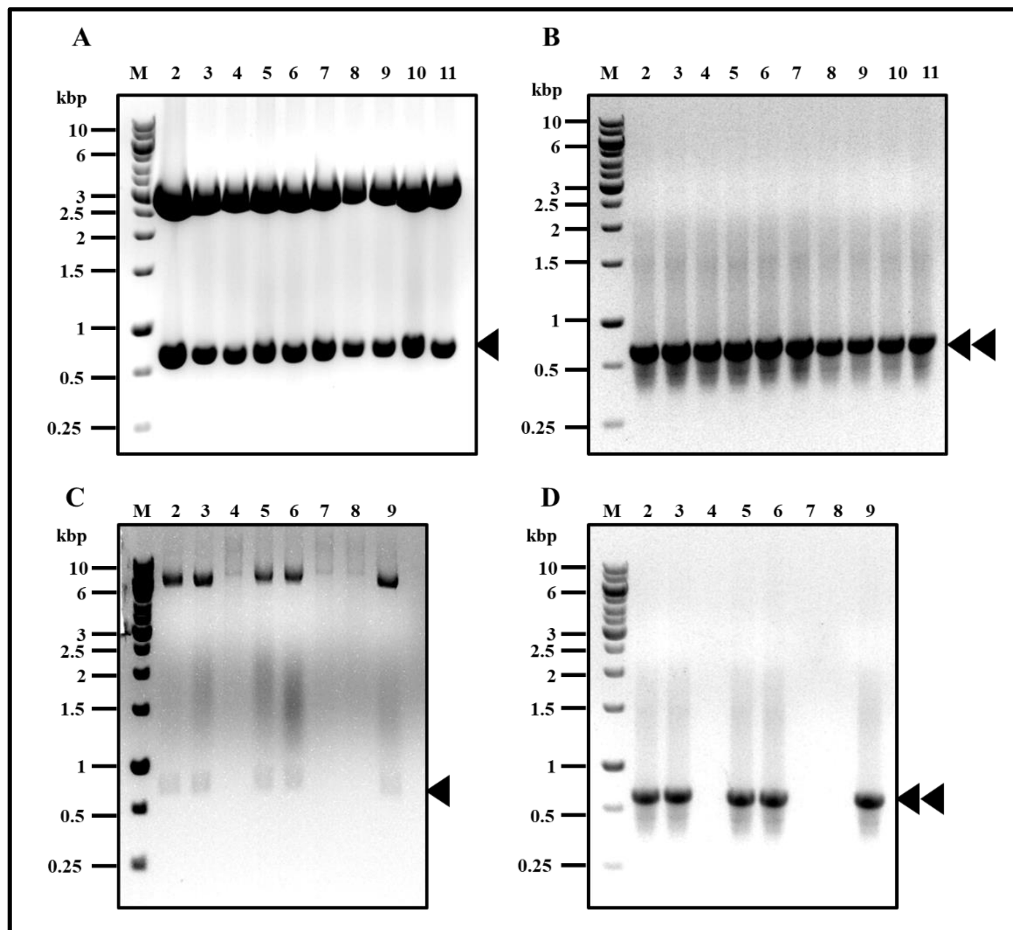

Figure 4.1. || Confirmation of recombinant Gp07 clones.

(A) Confirmation of pGp07-T clones by DNA double-digestion using the restriction enzymes NdeI and XhoI. The double-digested products show two individual bands approximately 3 kbp and 825 bp representing the vector and the insert, respectively (Lanes 2–11). (B) Confirmation of selected recombinants (pGp07-T) by PCR amplification. (C) Confirmation of pGp07 clones by DNA double-digestion using the restriction enzymes NdeI and XhoI. The double-digested products show bands approximately 825 bp representing the insert (Lanes 2–9). (D) Confirmation of selected recombinants (pGp07) by PCR amplification. All analysis were carried out on 1.5% agarose gel. M is the 1 kbp DNA ladder. Single arrowhead indicates the digestion product whereas PCR products were indicated by double arrow head.

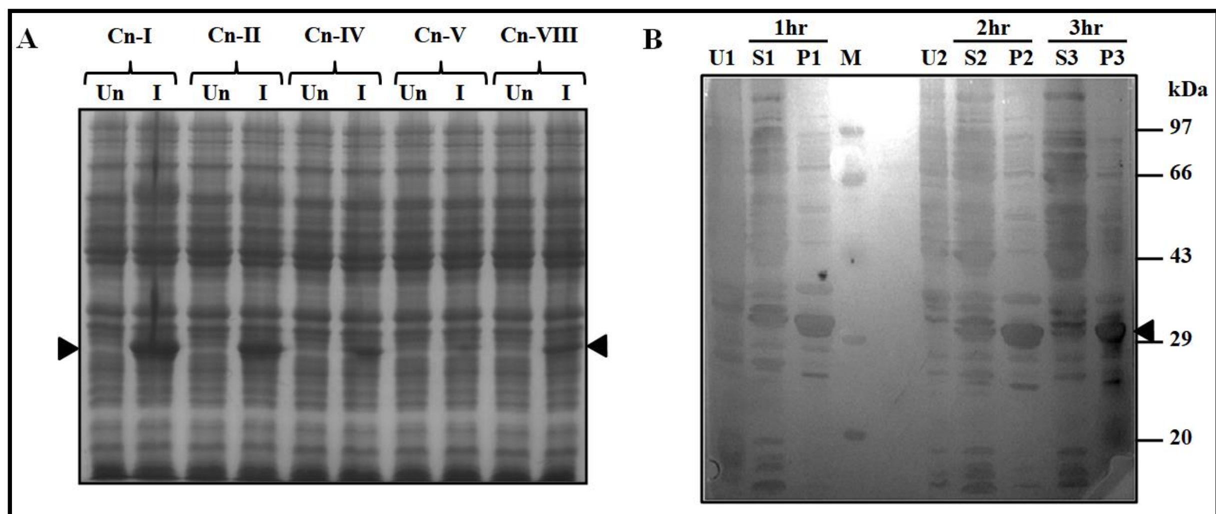

Figure 4.2. || Gp07 overexpression.
(A) Protein gel demonstrating heterologous expression of recombinant protein Gp07. The selected recombinants were over-expressed and analyzed in SDS-12% PAGE. Cn represented as construct number. (B) Optimization of time for overexpression and purification of Gp07. U indicates Un-induced, S indicates induced supernatant, P indicates induced pellet; Molecular masses of the marker (M) proteins (in kDa) were shown at the right side of the gel, and arrow head indicates Gp07. U1, U2 indicate uninduced control at 1hr and 2 hr respectively. S1, S2 and S3 indicate induced supernatant at 1 hr, 2hr and 3hr respectively. P1, P2 and P3 indicate induced pellet fraction at 1hr, 2hr and 3hr respectively.

4.3.3. **Large-scale protein expression and purification of His-tagged Gp07**

Cn-I was selected for large scale production of His-tagged Gp07. Large-scale protein production was performed in *E. coli* BL21(λDE3) cultured in 3 liters of LB media containing 50µg/ml kanamycin. The first stage of IMAC (Ni^{2+}-NTA) successfully enriched Gp07 from clarified cell lysates and the different fractions of washes and elutes were analyzed by SDS-12%PAGE (Figure 4.3.A). As is clear from the Figure 4.3.A, Gp07 could not be purified to homogeneity after the first IMAC and the presence of contaminating proteins are largely evident. Recombinant Gp07 obtained from the first IMAC stage was dialysed overnight at 4ºC with the modified dialysis buffer (20mM Tris pH 7.5, 500mM NaCl, 0mM Imidazole, 5% glycerol and 1mM EDTA). To remove proteins that bound non-specifically during the first IMAC stage, the dialyzed Gp07 was subjected to a second Ni^{2+} affinity chromatography step. In this step, most of non-specific proteins were found in the wash 1 fraction (W1 lane in Figure 4.3.B), and purified recombinant Gp07 was eluted from the resin with buffer containing 300 mM imidazole (E1-E9 lanes in Figure 4.3.C). A substantial amount of Gp07 was also found in the flow through fraction (F lane in Figure 4.3.B). Thus, a single band purification of the Gp07 was achieved as can be seen in the elution fraction (Figure 4.3.C). The different elution fractions were pooled together and dialyzed against dialysis buffer as per the

requirements of further experiments. The final concentration of the dialyzed recombinant protein was found to be ~3.2mg/3liters of culture estimated by Bradford protein estimation assay.

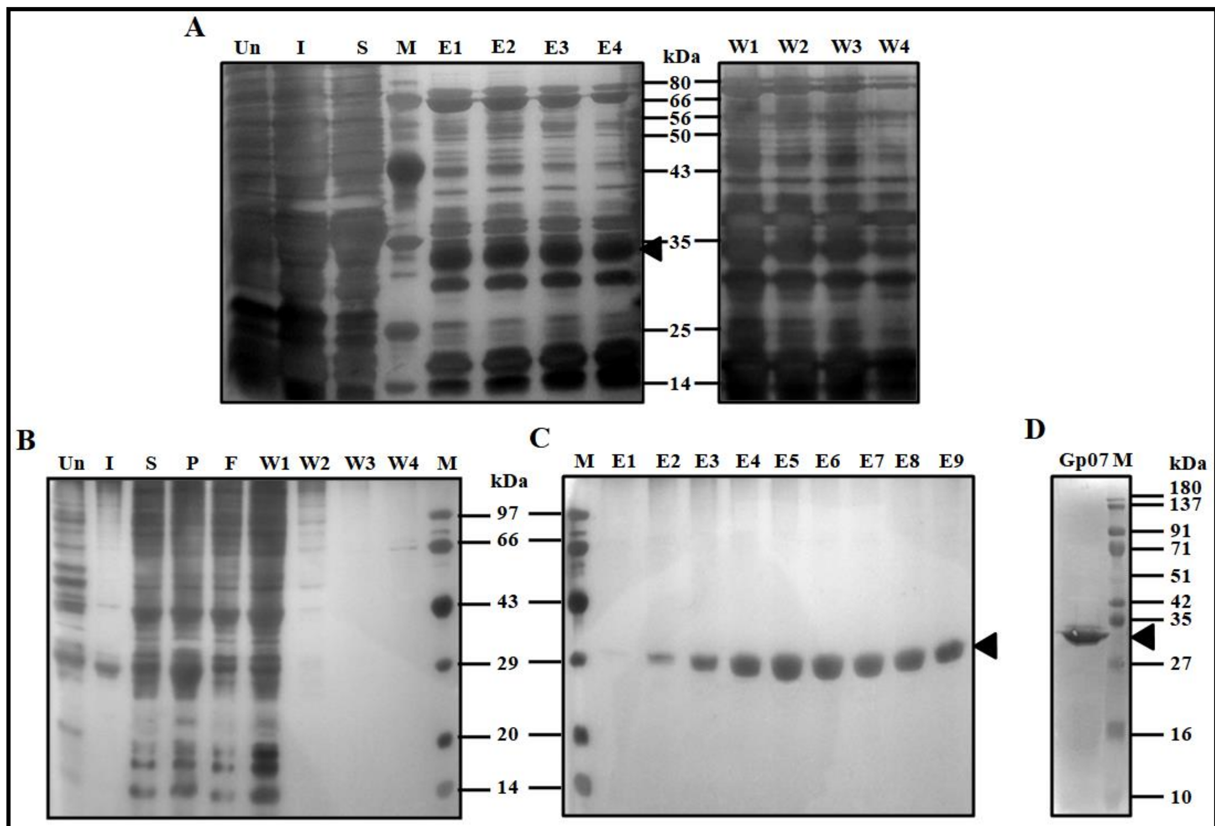

Figure 4.3. || Purification of Gp07, fractions analysed by SDS-12% PAGE.
(A) E indicate elution fractions (E1-E4), Molecular masses of the marker (M) proteins (in kDa) were shown on the right side of the gel, and arrow head indicate Gp07. (B) Purification of Gp07. All fractions were prepared from Gp07 over-expressed cell extract. Un-induced (Un), induced (I), supernatant (S), pellet (P), flow-thorough (F), washes (W1, W2, W3 and W4). (C) Elution fractions (E1-E9 after 2nd stage of IMAC purification) are loaded in different lanes. Arrowhead indicates Gp07. Molecular masses of the marker (M) proteins (in kDa) were shown. (D) Western blotting analysis of purified Gp07 protein expressed in *E. coli* BL21(λDE3) cells. Arrowhead denoted Gp07 that interacted with anti-his antibody. Molecular masses of the marker (M) proteins (in kDa) are shown at the right side.

4.3.4. **Oligomeric state of Gp07**

To gain information about the oligomeric status of Gp07 in solution, the protein (~5 μM) was subjected to analytical gel filtration chromatography. The Gp07 chromatogram primarily shows a single peak with the retention time of 24.08 min (Figure 4.4.A). In comparison with the retention time of some standard proteins, the elution volume of Gp07 indicates that its apparent molecular mass is 62.7 kDa (Figure 4.4.B). As the theoretical mass of Gp07 is 32.8 kDa, this protein mostly exists as a dimer in the aqueous solution. The data together indicates that repressor forms significant amount of dimers in solution at ~5 μM concentration. A glutaraldehyde-mediated cross-linking of Gp07 was performed as stated in the methodology. There is a formation of ~65 kDa protein species

in the presence of glutaraldehyde, suggesting the dimerization of Gp07 in the aqueous solution (Figure 4.4.C).

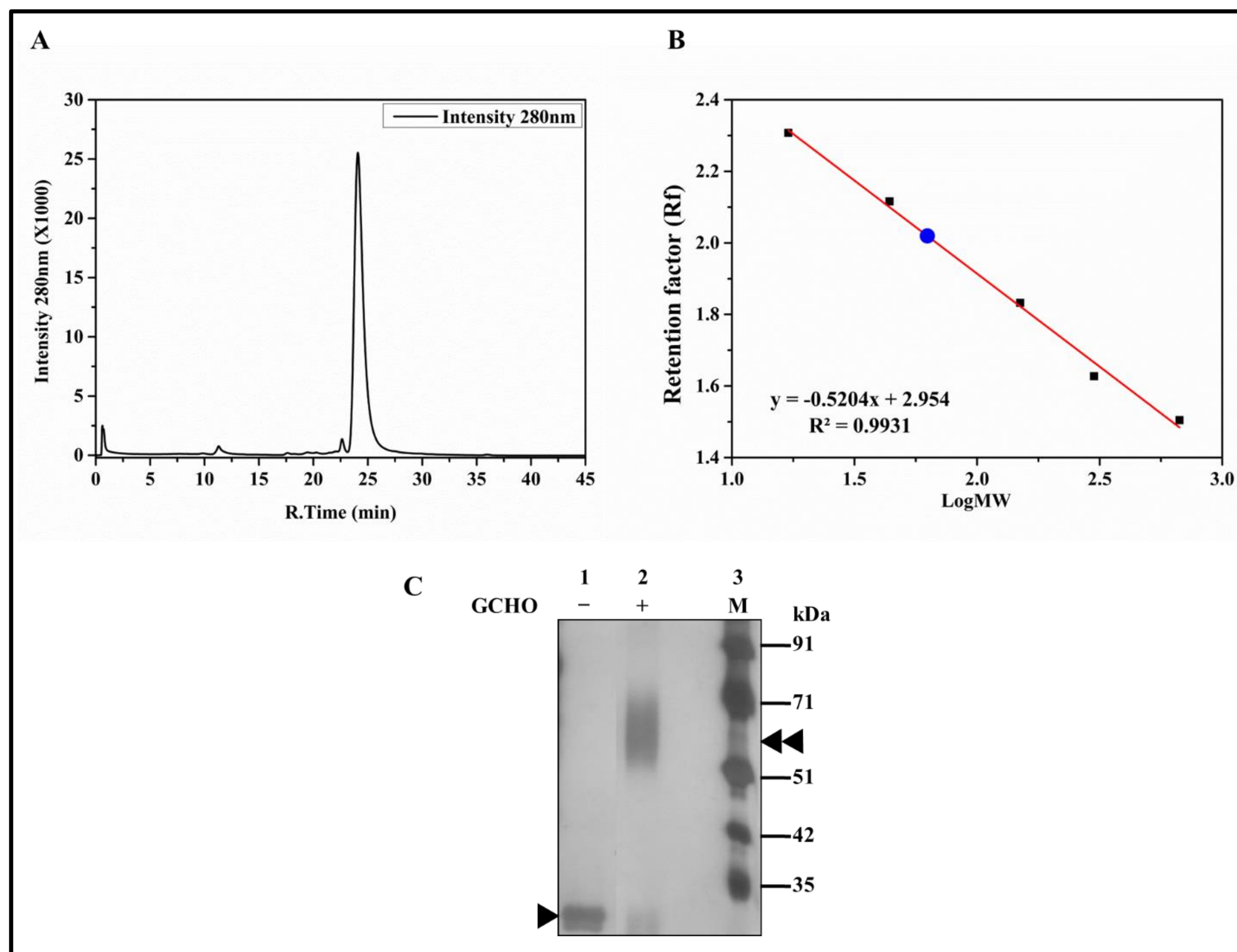

Figure 4.4. || Oligomerization analysis of Gp07 by gel filtration chromatography.
(A) Analytical size-exclusion chromatography of purified full-length Gp07 with a SEC-s2000 column (Phenomenex). (B) The Rf values of different marker proteins, determined from their elution profiles, were plotted against their respective molecular masses. The calibration curve is shown, where Log MW is the logarithm of the molecular weight and Rf is the retention factor. The blue dot shows the Log MW of Gp07. The equation resulting from this graph was used to determine the mass of Gp07. (C) Characterization of the oligomeric state of Gp07 by glutaraldehyde (GCHO) cross-linking. Gp07 dimer was determined in relation to molecular mass standards (M). Lane 1: Gp07 in absence of GCHO, lane 2: Gp07 in presence of GCHO and lane 3: molecular standard marker. Reaction mixtures were analyzed by 12% Tris-glycine SDS-PAGE. Dimer and monomer form of Gp07, indicate by single and double arrowhead respectively.

4.3.5. **Thermal denaturation of Gp07 monitored by CD spectroscopy**

The far UV CD results clearly indicates that the negative chirality of the CD signal decreases with rise in temperature (Figure 4.5.A). This occurrence is most noticeable between 35˚C and 45˚C. From 15°C to 35°C, the CD spectrum is dominated by α-helical structure while at higher temperatures (45°C-85°C) the CD spectra is dominated by β-sheet (anti-parallel) and random coil. It is well documented that rise in temperature normally disturbs the native conformation of proteins

and sponsor aggregation (Carpenter et al 1999). Thus, the aggregation of Gp07 is probably induced by the rise in temperature which is reflected in the loss in CD signal. Additionally, it is observed that at temperatures ranging from 65˚C-85˚C, the loss in CD signal reaches a plateau (Figure 4.5.B). Probably at temperatures at and above 65˚C there is a saturation in the aggregation process, leading to superimposition of the curves. The change in the CD data was best-fitted by a two-state process, and the midpoint temperature of transition (T) was 50.38 ± 1.9°C.

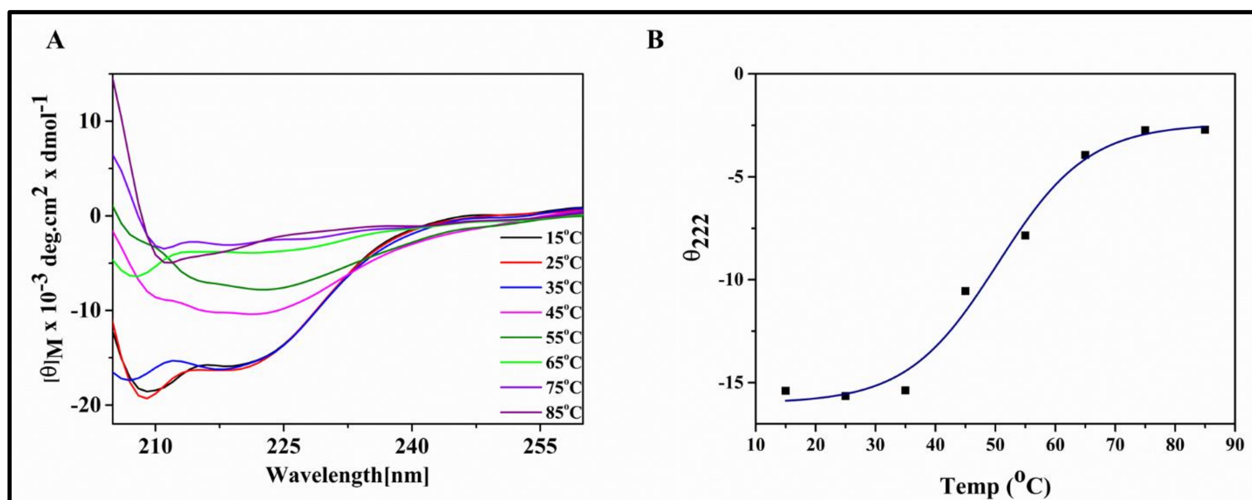

Figure 4.5. || Temperature-induced unfolding of Gp07.
(A) Far-UV CD spectra of Gp07 in the presence of indicated temperature. (B) Rising temperature leads to changes in the ellipticity at 222nm.

4.3.6. **GdnHCl mediated changes in structure of Gp07**

Intrinsic tryptophan fluorescence studies (λ_{ex} at 295 nm) were performed to study any changes in the architecture of Gp07 due to the effect of the GdnHCl. The fluorescence spectra after unfolding of Gp07 showed a shift in the emission maxima (λ_{max}) (Figure 4.6.A). This shift was more prominent with higher GdnHCl concentration. Out of four tryptophan residues in Gp07, one of the residues is present as the second amino acid from the amino terminal of the protein, and is not a part of the alpha helix or beta sheet (Figure 4.6.B). The other two tryptophans are approximately at the middle, one being at the 111th position (and is a part of the alpha helix) while the other is at the 216th position (is a part of the beta sheet). The last tryptophan residue is present at the carboxy terminal (250th position) of the protein (is a part of the beta sheet). These three tryptophan residues (at 111th, 216th and 250th position) possibly contribute to the red shift. A larger shift in λ_{max} for an unfolded protein indicates a complete exposure of a buried tryptophan upon unfolding. In case of Gp07, a large shift (from λ_{max}338 to λ_{max}354) with rising GdnHCl concentration is indicative of a complete exposure of the tryptophan residues upon unfolding of the protein.

Figure 4.6. || GdnHCl-induced unfolding of Gp07.

(A) Intrinsic Trp fluorescence spectra of Gp07 at 0-6 M GdnHCl. (B) Gp07 secondary structure predicted with the PSI-PRED server. The Gp07 secondary structure showed to be organized in coil regions interrupted with short beta sheets and alpha helices. The PSI-PRED model showed eleven alpha helices (pink barrels) and ten beta sheets (yellow arrows).

4.4. **Discussion**

This work reports the cloning, overexpression and purification the Phi11 Gp07 protein for the first time, in a heterologous *E. coli* system, as a histidine tagged variant. Due to the lethal effect of Gp07 overexpression upon the host cells (Chapter 3) (Das et al 2018), it is extremely difficult to overexpress and purify the protein.

This chapter describes, the optimization of the time of induction at which the protein appears in the soluble extract. Further, a two stage IMAC purification has been employed which leads to purification of the protein to homogeneity. The oligomeric status of the protein as well as the effect of different temperatures upon the secondary structure of Gp07 has also been investigated in this chapter. The status of the tryptophan residues (buried or exposed) has also been reported. Despite the comparatively low protein yields obtained here, the present work paves the way for the further biochemical and structural characterization of Gp07.

4.5. **References**

- Albrecht, C., (2008). Joseph R. Lakowicz: Principles of fluorescence spectroscopy. *Analytical and Bioanalytical chemistry*, *390*(5), pp.1223-1224.
- Archer, G.L., (1998). Staphylococcus aureus: a well-armed pathogen. *Reviews of Infectious Diseases*, *26*(5), pp.1179-1181.
- Brown, D.T., Brown, N.C. and Burlingham, B.T., (1972). Morphology and physical properties of Staphylococcus bacteriophage P11-M15. *Journal of virology*, *9*(4), pp.664-671.
- Buchan, D.W., Minneci, F., Nugent, T.C., Bryson, K. and Jones, D.T., (2013). Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucleic acids research*, *41*(W1), pp.W349-W357.
- Carpenter, J.F., Kendrick, B.S., Chang, B.S., Manning, M.C. and Randolph, T.W., (1999). [16] Inhibition of stress-induced aggregation of protein therapeutics. In *Methods in enzymology*(Vol. 309, pp. 236-255). Academic Press.
- Creighton, T.E. ed., (1997). *Protein structure: a practical approach* (No. 174). Oxford university press.
- Das, A., Biswas, S. and Biswas, M., (2018). Expression of Phi11 Gp07 Causes Filamentation in Escherichia coli. *The open microbiology journal*, *12*, p.107.
- Das, M., Ganguly, T., Bandhu, A., Mondal, R., Chanda, P.K., Jana, B. and Sau, S., (2009). Moderately thermostable phage Φ11 Cro repressor has novel DNA-binding capacity and physicochemical properties. *BMB reports*, *42*(3), pp.160-165.
- Das, M., Ganguly, T., Chattoraj, P., Chanda, P.K., Bandhu, A., Lee, C.Y. and Sau, S., (2007). Purification and characterization of repressor of temperate S. aureus phage Φ11. *BMB Reports*, *40*(5), pp.740-748.
- Iandolo, J.J., Worrell, V., Groicher, K.H., Qian, Y., Tian, R., Kenton, S., Dorman, A., Ji, H., Lin, S., Loh, P. and Qi, S., (2002). Comparative analysis of the genomes of the temperate bacteriophages φ11, φ12 and φ13 of Staphylococcusaureus 8325. *Gene*, *289*(1), pp.109-118.
- Iyer, L.M., Koonin, E.V. and Aravind, L., (2002). Extensive domain shuffling in transcription regulators of DNA viruses and implications for the origin of fungal APSES transcription factors. *Genome biology*, *3*(3), pp.research0012-1.
- Lemire, S., Figueroa-Bossi, N. and Bossi, L., (2011). Bacteriophage crosstalk: coordination of prophage induction by trans-acting antirepressors. *PLoS genetics*, *7*(6), p.e1002149.
- Liu, T., Renberg, S.K. and Haggård-Ljungquist, E., (1998). The E protein of satellite phage P4 acts as an anti-repressor by binding to the C protein of helper phage P2. *Molecular microbiology*, *30*(5), pp.1041-1050.
- Micsonai, A., Wien, F., Bulyáki, É., Kun, J., Moussong, É., Lee, Y.H., Goto, Y., Réfrégiers, M. and Kardos, J., (2018). BeStSel: a web server for accurate protein secondary structure prediction and fold recognition from the circular dichroism spectra. *Nucleic acids research*.
- Riedel, H.D., Heinrich, J., Heisig, A., Choli, T. and Schuster, H., (1993). The antirepressor of phage P1 Isolation and interaction with the C1 repressor of P1 and P7. *FEBS letters*, *334*(2), pp.165-169.

- Shearwin, K.E., Brumby, A.M. and Egan, J.B., (1998). The Tum protein of coliphage 186 is an antirepressor. *Journal of Biological Chemistry*, *273*(10), pp.5708-5715.