Bacterial Expression and Purification of Human Papillomavirus Type 18 L1

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Abstract The human papillomavirus (HPV) 18 L1 gene, which encodes the L1 major capsid protein, was isolated from a female patient in Pusan, Korea Republic and was cloned into pGEX-4T-1 vector. The HPV-18L1 gene was expressed in *Escherichia coli* as a fusion protein with a glutathione-S-transferase (GST) tag. The soluble recombinant fusion protein, GST-18 L1 fusion, was isolated to high purity. HPV-18 L1 was purified from the GST-18 L1 fusant after biotinylated thrombin cleavage, and then the treated thrombin was removed serially using streptavidin conjugated resin. The purified HPV-18 L1 was confirmed by western blotting using a rabbit anti-denatured papillomavirus polyclonal antibody. The virus-like particles (VLP) from the purified full-length 18 L1 protein without any extra amino acid sequences was observed through the analysis of the electron microscope. This is the first study to report the expression and purification of HPV-18 L1 in *E. coli*. This expression and purification system offers a simple method of expressing and purifying HPV L1 protein, and could potentially be an effective route for the development and manufacturing of highly purified HPV-18 L1-based cervical cancer vaccines. © KSBB

Keywords: human papillomavirus, bacterial expression, purification, L1 major capsid protein, HPV type 18

INTRODUCTION

Human papillomavirus (HPV) is a member of the papoviridae family of viruses, which are highly relevant human pathogens. HPV is a non-enveloped, small double-stranded DNA virus containing a circular genome of approximately 8,000 base pairs [1]. Native virions of HPV are icosahedral structures that are 50~60 nm in diameter and consist of 72 capsomers, each of which is composed of L1 molecules [2]. There are more than 200 known distinct types of HPV that have been identified based on distinct genomic differences in DNA sequence data and these different types can infect a range of different epithelial surfaces, including hands, feet, and the genital region [3]. Low-risk subtypes, such as 6, 11, 42, 43, and 44, are the causative agent of warts and condyloma acuminata (genital warts), while the high-risk subtypes, such as HPV 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70, have been shown to be the causative agent of cervical cancer [4,5].

Four types, 16, 18, 31, and 45, are most often found within malignant cells of cervical cancers [6,7]. HPV 16 is the cause of about 54% of invasive cervical cancers and HPV 18 is responsible for about 17% worldwide [8]. In addition, approximately 90% of all genital warts cases are caused by HPV 6 or HPV 11 [9].

Heterogenic eukaryotic expression systems have recently been employed to generate virus-like particles (VLPs). The major capsid protein L1 has a relative molecular weight of 55 kDa by reducing SDS-PAGE [10]. Recombinant VLPs have been expressed in animal cells [11], insect cells [12,13], yeast [14], and bacterial cells [15,16], and represent the leading candidate vaccines for preventing cervical cancer [17].However, the use of bacterial expression systems offers distinct the advantages in relation to vaccine development, including cost-effective VLP production, high levels of product yield, and relative ease of purification [18,19]. A number of studies have reported successful expression of HPV L1 proteins in bacteria [10,20]. However, until now, there has been no report on the expression, purification, and VLP formation of HPV 18 L1 expressed in bacteria, even though this has been demonstrated in several other expression systems, such as animal [21], insect [22], and yeast [23].

In this study, we report a simple method for bacterial expression and purification of HPV-18 L1. The L1 gene encoding the major capsid protein from HPV type 18 was isolated from a female patient, cloned into bacterial expression vector,

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pGEX-4T-1, and expressed in *Escherichia coli*. The recombinant L1 protein was purified using glutathione sepharose and streptavidin conjugated resins, and confirmed by western blotting analysis with a rabbit anti-denatured PV polyclonal antibody. In addition, VLPs from the purified 18 L1 proteins were observed.

MATERIALS AND METHODS

Materials

Viral DNA was extracted from the cervical cell samples of a female patient, Pusan in Korea, using a DNA extraction kit, Wizard® Genomic DNA Purification Kit which was purchased from Promega (WI, USA). The HPV-18 L1 gene was amplified by polymerase chain reaction (PCR) using viral genomic DNA as the template. Primers were synthesized at Bioneer (Deajeon, Korea). DNA sequencing was performed by Genotech (Deajeon, Korea). The pGEM-T Easy and pGEX-4T-1 vectors were purchased from Promega (WI, USA) and Amersham Biosciences (NJ, USA), respectively. *E. coli* host strain DH5α and BL21 (DE3) were purchased from Real Biotech (Seoul, Korea), and used as the host strains for cloning and expression of the HPV-18 L1 gene, respectively. *E. coli* was grown in Luria-Bertani (LB) medium at 37°C. Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from Amresco Inc. (OH, USA). The QIAEX® II Gel Extraction Kit, which was used for DNA purification, was purchased from QIAGEN GmbH (Hilden, German), *Ex-Taq* polymerase, which was used for PCR amplification, T4 DNA ligase, and restriction enzymes were purchased from TaKaRa (Shiga, Japan). Glutathion Sepharose 4B was purchased from GE Healthcare (Sweden). Rabbit anti-papillomavirus polyclonal antibody was obtained from Dr. S. J. Ghim (Department of Pathology, Georgetown University Medical Center). Goat anti-rabbit immunoglobulin G (IgG) conjugated with alkaline phosphatase (AP) and AP detection reagent were purchased from Novagen (Darmstadt, Germany). All other reagents were local products of analytical grade.

Purification of Viral DNA and Plasmid Constructions

DNA was extracted from cervical cell samples of patients using a DNA extraction kit (Promega). The full-length HPV-18 L1 DNA sequence was obtained by PCR amplification with a forward primer, 5′-CCCGAATTCATGTGCCTGTA-TACACGGGTC-3′ containing the *Eco*RI restriction enzyme site at the initiator methionine codon, and a reverse primer 5′-CCCCTCGAGTTACTTCCTGGCACGTACACG-3′, containing the *Xho*I restriction enzyme site at the 3′ terminal sequence (the underlined sequences are the restriction enzyme sites). The HPV-18 L1 genes, which are approximately 1.7 kb, were routinely amplified using the following reaction conditions: 94°C for 1 min, 53°C for 1 min, 72°C for 2 min, and 72°C for 2 min, and 30 cycles of PCR. The PCR-amplified fragment was purified with the QIAEX® II Gel

Extraction Kit (QIAGEN). The PCR amplicon was ligated into the plasmid pGEM-T Easy (Promega). *E. coli* DH5α clones harboring pGEM-T Easy-HPV-18 L1 were selected and then HPV-18 L1 DNA sequencing was performed on DNA samples isolated from three individual colonies to verify HPV-18 L1 DNA sequence. To generate pGEX-4T-1-HPV-18 L1 plasmid, the plasmid pGEM-T Easy carrying HPV-18 L1 was digested with *Eco*RI and *Xho*I and then the *Eco*RI and *Xho*I fragment of HPV-18 L1 was ligated to pGEX-4T-1 and transformed to *E. coli* BL21 (DE3). Ampicillin (100 µg/mL) was used when necessary.

Recombinant L1 Expression and Purification

E. coli BL21 (DE3) clones harboring the expression plasmid, pGEX-4T-1-HPV-18 L1, were grown in 10 mL LB medium with ampicillin (100 µg/mL) overnight. The overnight cell culture was inoculated to one liter of LB medium and grown at 37°C to the mid-exponential stage (A_{600} of approximately 0.5). The expression of the GST fusion 18 L1 was then induced by the addition of 0.1 mM IPTG followed by incubation for 12 h at 18°C. The induced cells were harvested, washed with phosphate-buffered saline (PBS), pelleted by centrifugation, and then stored at −70°C until further use. Bacteria cells from the one liter culture were thawed in 30 mL of ice-chilled PBS. The cell suspension was pulse-sonicated at 1 min intervals for 30 min. The soluble and insoluble fractions were separated by centrifugation at 12,000 $\times g$ for 20 min at 4°C. The insoluble fraction was re-extracted with 10 mL of the same buffer. 100 µL (50 µL of bed volume) of the 50% slurry of Glutathion Sepharose 4B (Amersham Biosciences) equilibrated with PBS was added to the soluble fraction and it was incubated for 30 min at room temperature with agitation using an end-over-end rotator. The medium containing the adsorbed fusion protein was sedimented by centrifugation at 500 $\times g$ for 5 min. The supernatant was carefully discarded and then washed with 10 mL of PBS by using inverting to mix. These steps were repeated for three times. The biotinylated thrombin (5 units) (Novagen) and 30 µL of 10X thrombin cleavage buffer (200 mM Tris-HCl pH 8.4, 1.5 M NaCl, and 25 mM $CaCl_2$) were added to the sediment to a total volume of 265 µL. The mixture was incubated for 3 h at room temperature with agitation using an end-over-end rotator, followed by centrifugation at 500 $\times g$ for 5 min. The supernatant from the mixture was carefully obtained and the sediment was re-extracted with 300 µL of the same buffer. Eighty µL (40 µL of bed volume) of streptavidin-conjugated resin (Novagen) was added to a total volume of 500 µL of the supernatant and incubated for 15 min at room temperature with agitation using an end-over-end rotator. This was followed by centrifugation at 500 $\times g$ for 5 min. Purification steps were monitored using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie Brilliant Blue R-250 (Bio-Rad) staining. Protein concentration was determined using the Bradford method [24] with bovine serum albumin as standard by measuring the absorbance of solutions at 595 nm, UV visible Spectrophotometer.

Western-blot Analysis

The SDS-PAGE gels were electrotransferred onto polyvinyl difluoride (PVDF) membranes (Roche, Germany) for 90 min at 0.2 A using a Bio-Rad transblot apparatus. The PVDF membranes were activated, according to the manufacture's instruction, before use. The membrane was incubated with a blocking solution [1X Tris-buffered saline (TBS; 150 mM NaCl, 50 mM Tris-Hcl, and pH 7.5), 5% fat-free milk powder, and 0.1% Tween-20] for 1 h at RT. The membrane was serially washed two times with TBST (TBS buffer with 0.1% Triton-X) for 10 min and one time with TBS for 10 min. The membrane was then incubated with rabbit anti-papilomavirus (PV) polyclonal antibody in blocking solution for a 1 h. The membrane was washed serially two times with TBST for 10 min and one time with TBS for 10 min and then incubated with Goat anti-rabbit IgG-alkaline phosphatase (AP) conjugate (Novagen, Germany) in blocking solution for 1 h. After washing five times with TBST, each for 10 min, the membrane was visualized using an AP detection reagent (Novagen, Germany).

Electron Microscopy

The L1 protein was analyzed with a Tecnai G2 spirit transmission electron microscope (FEI, USA) using the negative staining method at the Korea Basic Science Institute (Korea). The purified 18 L1 protein sample was applied to glow-discharged carbon-coated copper grids. After allowing the protein to absorb for 2 mins and blotting off buffer-protein solution onto Whatman paper, then the protein on the grids were stained with 1% (w/v) uranyl acetate (UrAc) for 1 min. Then it was blotted off UrAc. These results were recorded with the Technai G_2 Spirit Twin microscope (FEI, USA) at an acceleration voltage of 120 kV.

RESULTS

Isolation and Cloning of HPV-18 L1 from a Female Patient

The full-length HPV-18 L1 DNA was amplified from a sample obtained from a female patient using a primer set that was designed in this study. The conserved regions in the designed primers, which introduced an *Eco*RI restriction enzyme site for the forward primer and an *Xho*I restriction enzyme site for the reverse primer, were selected from the alignment of HPV-18 L1 sequences registered in GenBank. The PCR amplicon from the DNA sample was cloned into a pGEM-T Easy vector after gene purification. The 1,707 bps HPV-18 L1 DNA sequence was amplified (Fig. 1B) and confirmed by DNA sequencing. The DNA sequence of HPV-18 L1 was then registered in GenBank (accession number EU834744). The DNA sequence analysis revealed that the HPV-18 L1 DNA produced in this study had the highest level of sequence similarity (99.8%) to human papillomavirus type 18 L1 (EF202145) registered on GenBank. There
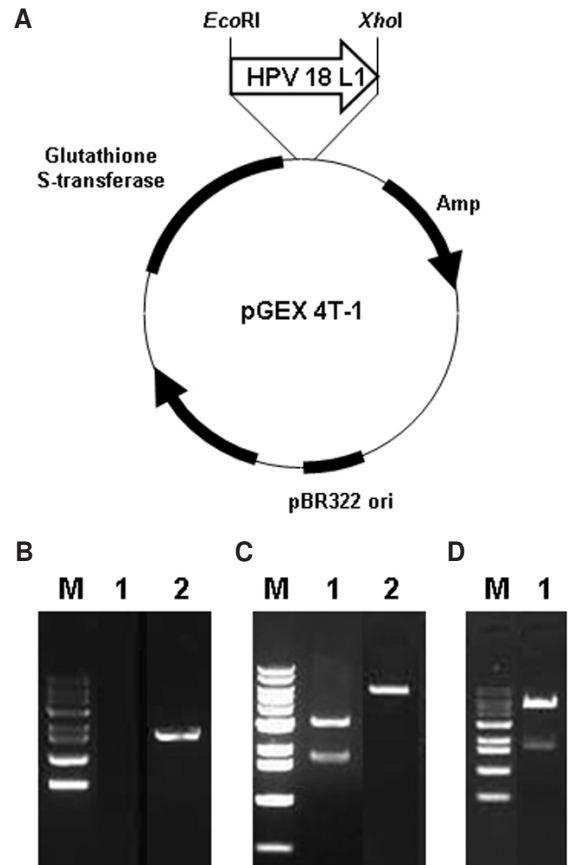

Fig. 1. Isolation and cloning of HPV-18 L1 and construction of the expression vector pGEX-4T-1-18 L1. (A) Construction of pGEX-4T-1-HPV-18 L1. (B) PCR product of 18 L1. Lane M, DNA marker; lane 1, negative control; lane 2, PCR product of HPV 18 L1. (C) Restriction endonuclease analysis of the cloning vector pGEM-TE-18 L1 and expression vector pGEX-4T-1. Lane M, DNA marker; lane 1, pGEM-TE-18 L1 digested with *Eco*RI and *Xho*I; lane 2, pGEX-4T-1 digested with *Eco*RI and *Xho*I. (D) Restriction endonuclease analysis of the expression vector pGEX-4T-1-18 L1. Lane M, DNA marker (10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.6, 1.0, and 0.5 kb); lane 1, pGEX-4T-1-18 L1 digested with *Eco*RI and *Xho*I.

were only two different positions (position 68 and 531) that differed between these DNA sequences. The difference at position 68 had the largest impact on the resulting amino acid sequence since it changed the encoded amino acid from a proline to a histidine (at position 23 in amino acid sequence) (data not shown).

Construction of HPV-18 L1 Expression Plasmids

After digestion with *Eco*RI and *Xho*I, the restriction fragments of HPV-18 L1 were obtained from the plasmid pGEM-T Easy-HPV-18 L1 and cloned into the pGEX-4T-1 *Eco*RI and *Xho*I digested bacterial expression vector (Figs. 1A, 1C, and 1D).
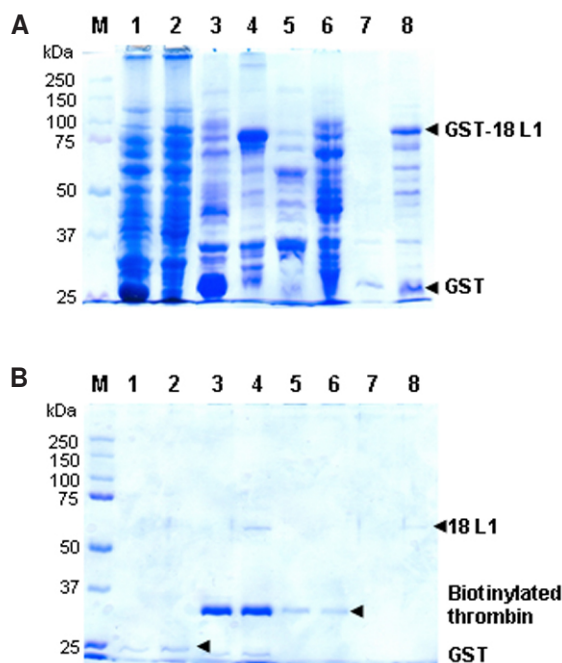
Fig. 2. SDS-PAGE analysis of the expression and purification of the GST-18 L1 fusion and 18 L1 proteins in *E. coli* with Coomassie blue staining. (A) The expression and purification of GST-18 L1 fusion protein. Lane M, protein marker; lane 1, whole cell lysate from the culture of *E. coli* harboring pGEX-4T-1 as the control; lane 2, whole cell lysate from the culture of *E. coli* harboring pGEX-4T-1-18 L1; lane 3, insoluble fraction from *E. coli* harboring pGEX-4T-1; lane 4, insoluble fraction from *E. coli* harboring pGEX-4T-1-18 L1; lane 5, soluble fraction from *E. coli* harboring pGEX-4T-1; lane 6, soluble fraction from *E. coli* harboring pGEX-4T-1-18 L1; lane 7, purified GST-bound resin; lane 8, purified GST-18 L1 fusion-bound resin. (B) 18 L1 purification from the GST-18 L1 fusion protein. Lane M, protein marker; lane 1, the sediment from the purified GST-bound resin after biotinylated thrombin treatment; lane 2, the sediment from the purified GST-18 L1 fusion-bound resin after biotinylated thrombin treatment; lane 3, soluble fraction from the purified GST-bound resin after biotinylated thrombin treatment; lane 4, soluble fraction from the purified GST-18 L1 fusion-bound resin after biotinylated thrombin treatment; lane 5, the sediment after streptavidin treatment of the soluble fraction (sample of lane 3); lane 6, the sediment after streptavidin treatment of the soluble fraction (sample of lane 4); lane 7, final eluent from sample of lane 3; lane 8, final eluent from sample of lane 4.

Expression of Fusion Protein

To optimize the expression condition, expression temperature, and IPTG concentration for induction were tested (data not shown). A final concentration of 0.1 mM IPTG for induction and further incubation for 12 h at 18°C, GST fusion-protein resulted in the highest level of expression. Consequently, these conditions were used throughout this study for

Table 1. Purification of recombinant HPV-18 L1 protein

Purification step	Protein (mg)	HPV 18 L1 protein (mg)	Purification (fold)	Yield (%)
Cell extract	105.6	-	1	100
Glutathion-sepharose	24.2	2.58[a]	10.6	22.9
Streptavidine agarose	2.71	0.94[b]	105.6	0.89

[a]GST fused HPV-18 L1, [b]HPV-18 L1.

expression and SDS-PAGE analysis. The expression plasmids pGEX-4T-1-HPV-18 L1 used for the production of the GST fusion protein and empty plasmids pGEX-4T-1 used as a control were transformed into the *E. coli* BL21 (DE3). After IPTG induction, the GST fusion protein was shown to have a molecular weight of approximately 86 kDa by SDS-PAGE, while only a 27 kDa band was detected in the GST control (Fig. 2).

Purification of HPV-18 L1 Protein

When the HPV-L1 protein was expressed at 18°C after IPTG induction, the GST-18 L1 fusants were found in both the insoluble cell pellet and the soluble fraction of the cell lysate. However, when expressed at 37°C, the GST-18 L1 fusants were accumulated only in the insoluble fraction. As glutathione-Sepharose resin was added to the soluble fraction, the GST fusion proteins were retained on it (Fig. 2).

The biotinylated thrombin digestion of the glutathione conjugated resin-bound GST-18 L1 protein released 18 L1 protein from the resin, and the 18 L1 protein band shifted to approximately the 59 kDa (Fig. 2B). A thrombin band was also observed in the SDS-PAGE gel as an additional intense band at 35 kDa (Fig. 2B). In order to remove the thrombin and purify the 18 L1 protein from the eluent, streptavidin-conjugated resin was added to the eluent to capture the biotinylated thrombin and then the mixture was incubated for an additional 10 min. In the sediment, the captured thrombin was seen on SDS-PAGE (Fig. 2B, lanes 5 and 6). The amount of the captured thrombin was smaller than that of the eluent before addition of the steptavindin-agarose. This difference in thrombin concentration most likely occurred because of auto-proteolysis during incubation at room temperature (Fig. 2B). The purified full-length L1 protein of HPV 18 migrated on SDS-PAGE with an apparent molecular mass of 59 kDa (Fig. 2B). A summary of the purification steps is presented in Table 1.

Western-blot Assay

The purified full-length 18 L1 protein was confirmed by western-blot analysis using rabbit anti-PV polyclonal antibody (Fig. 3). Under these conditions of purification, approximately 0.4~0.8 mg of 18 L1 was obtained from one liter of bacterial culture.

Electron Microscopy Detecting Self-assemble of HPV-18 L1 Proteins into Virus-like Particle (VLP)
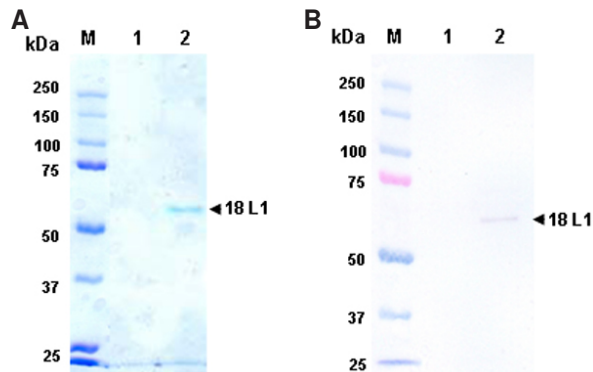
The purified 18 L1 protein were pooled and used for the

Fig. 3. Western-blotting analysis of the 18 L1 proteins expressed in *E. coli* after purification. (A) SDS-PAGE. Lane M, protein marker; lane 1, purified eluent obtained from the culture of *E. coli* harboring pGEX-4T-1; lane 2, purified eluent obtained from the culture of *E. coli* harboring pGEX-4T-1-18 L1. (B) Western-blotting, the purified 18 L1 was detected with the rabbit anti-papillomavirus polyclonal antibody. lane M, protein marker; lane 1, purified eluent obtained from the culture of *E. coli* harboring pGEX-4T-1 as the negative control; lane 2, purified eluent obtained from the culture of *E. coli* harboring pGEX-4T-1-18 L1.
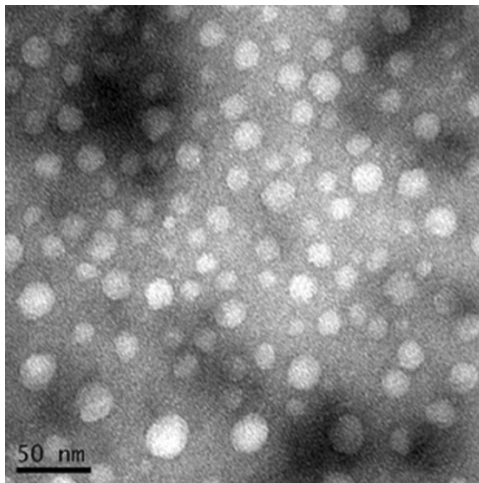

Fig. 4. Purified HPV 18 L1 examined by transmission electron microscopy. Bar, 50 nm.

analysis of the transmission electron microscopy. The result from electron microscopy indicated the presence of VLP-like ordered particles. The HPV-18 L1 proteins were self-assembled into VLP ranging in diameter from 25 nm to 35 nm, which is smaller than those of papillomavirus virions (55 nm) (Fig. 4).

DISCUSSION

HPV 18 is a critical viral pathogen that causes cervical

cancer together with HPV 16. This is especially true after cervical intraepithelial neoplasia (CIN) 3, where HPV 16 is the cause of about 54% of invasive cervical cancers and HPV 18 is the cause of about 17% worldwide [8]. In this study, we have successfully expressed a HPV 18 L1 gene obtained from a female Korean patient and purified the resulting L1 protein. The full-length 1,707 bp HPV 18 L1 gene was amplified and the DNA sequence was confirmed (GenBank accession number EU834744).

The full-length 18 L1 gene was cloned into the bacterial expression vector, pGEX 4T-1. The expressed GST-18 L1 fusion protein was found in the insoluble aggregates at 37°C (data not shown). However, the expressed GST-18 L1 fusion protein accumulated into the soluble fraction of cell lysates at 18°C (Fig. 2). Several other studies have reported the insoluble aggregation of GST-16 L1 protein when the expression was induced at 37°C [25,26]. It was reported in protein expression of *E. coli*, the cold condition can improve the solubility of protein [27]. Chen *et al*. [28] reported that the GST fusion proteins of 16 and 11 accumulated in soluble fraction at room temperature, which is similar to the results obtained in this study. The GST-18 L1 fusion proteins had a molecular weight of approximately 86 kDa by SDS-PAGE (Fig. 2). It is well known that the GST fusion full-length HPV 16 L1 protein is approximately 82 kDa [25]. The full-length HPV 18 L1 protein (1,707 bps and 569 amino acids) is longer than 16 L1 (1,596 bps and 532 amino acids) in DNA and amino acid sequences, respectively. We tried to decrease non-specific bands, which occurred from the glutathione-conjugated resins, through extra washing steps with PBS, but the background did not decrease (Fig. 2). Fang and Ewald [29] have reported that the fusion protein can be purified to near homogeneity from crude cell lysates by a single affinity chromatography step, but in some case there are still problems and extra purification steps are required.

Glutathione-conjugated resin-bound GST-18 L1 fusant was treated with biotinylated thrombin for 3 h to release the 18 L1 protein from the fusant (GST remained on the glutathione-conjugated resin). The released 18 L1 and thrombin were monitored by SDS-PAGE as shown in Fig. 2. Under these conditions the background significantly decreased in the eluent after thrombin treatment, and most of non-specific material was shown in the sediment (Fig. 2B). In order to remove the biotinylated thrombin, the eluent was treated with streptavidin-conjugated resin to selectively capture biotinylated thrombin. The thrombin was effectively removed using this treatment (Fig. 2).

In this study, glutathione-coupled resin and streptavidin-conjugated resin were used for purification of HPV-18 L1 protein and these resins are known to have a high specificity to GST and biotin, respectively [28]. In addition, these two independent resin-capturing methods effectively reduced the level of impurities. The purification method used in present study was simple as one-step without any extra sequence like GST for HPV-18 L1 protein. We expect that it could apply to purify various proteins which do not permit extra amino acid sequences on them for specific purposes.

The VLP from the purified full-length 18 L1 protein with-

out any extra amino acid sequences in both of N- and C-terminal regions was observed through the analysis of the electron microscope. The sizes of 18 L1 VLP-like particles, approximately 25~35 nm, were slightly smaller than human papillomavirus virion, approximately 50~55 nm in diameter. This result suggests that the *E. coli*-produced recombinant L1 protein consisted of VLPs as previously described by McCarthy *et al*. [30]. Several previous studies have reported the *in vitro* assembly of bacterially expressed HPV L1 proteins into VLPs [28,31]. However, in those studies, the L1 proteins were accumulated in the cell as insoluble aggregates, which could block the assembly of L1 into VLPs *in vivo*. In addition, thin-section electron microscopy results did not indicate that the expressed HPV-16 L1 formed VLP structures in *E. coli* cells, and a renaturation process was required to restore the native folding of the protein and the ability of assembly VLPs [31]. However, in this study, it was detected to form VLP from the purified 18 L1 proteins without any renaturation process.

Recently, the expression and purification of HPV-18 L1 were reported using baculovirus expression system [32] and *Saccharomyces cerevisiae* expression system [25]. However, the use of bacterial expression systems offers distinct the advantages in relation to vaccine development, including cost-effective VLP production, high levels of product yield, and relative ease of purification. In addition, bacterial expression system is more convenient than any other systems, cell growth rapidity, simplicity of growth medium, low cost of growth medium, high expression level, and so on, even though it has limitation on posttranslational modification [33].

At present, some prophylactic HPV vaccines, which were expressed in yeast and baculovirus expression systems, have been commercialized for use in humans. However, the cost of the expression and purification in these systems are very high, in addition, the production efficacy is lower than that of bacteria. In order to overcome these problems, we have developed a bacterial expression system that is suitable for industrial production of HPV vaccine candidates.

It has been well established that L1 proteins from type 16, 11, and 6, which were expressed in different systems, self-assemble into VLPs. In previous studies 16 L1 protein was expressed in insect [12], yeast [14], plants [34], and bacteria [16,26,31]. However, only a few studies have reported expression of 18 L1 in different expression systems, including insect cell [22,35] and *S. cerevisiae* [23]. Interestingly, until now, there has been no report of the expression and purification of HPV-18 L1 protein in bacteria.

In this study, we isolated the HPV-18 L1 gene from a female Korean patient and cloned it into a bacterial expression vector. The bacterial expressed HPV-18 L1 protein was purified as in a soluble GST-18 L1 fusion form and 18 L1 protein was released from the fusant. The presence of the HPV-18 L1 protein was confirmed through western blot assay. Finally, VLPs from the purified 18 L1 proteins were observed. The expression and purification method described here should be useful for establishing HPV-18 L1-based cervical cancer vaccine design and offers a convenient method of manufacturing of highly purified HPV vaccines.

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