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شناسایی گروه غالب ویروس اس سیب زمینی (PVS) در ایران و بیان ژن پوشش پروتئینی آن

Identification of dominant isolate of *Potato virus S* in Iran and heterologous expression of its coat protein

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چکیده

بیان ژن ویروس‌ها در باکتری برای مطالعه پروتئین ویروس‌ها و تولید آنتی‌بادی اختصاصی مورد استفاده قرار می‌گیرد. در این پژوهش، نمونه‌های سیب‌زمینی از هشت استان ایران جمع‌آوری شده و ردیابی ویروس *Potato virus S*، به عنوان یکی از مخرب‌ترین ویروس‌های سیب‌زمینی، بوسیله داس-الیزا و آرتی-پی‌سی‌آر انجام گرفت. تعیین گروه غالب با توجه به توالی ژن پوشش پروتئینی و پس از تجزیه فیلوژنی انجام شد. ژن کامل CP تکثیر و همسانه‌سازی شده و مورد توالی‌یابی قرار گرفت، سپس از پلاسمید pJET1.2/blunt برش داده شده و به پلاسمید بیان pET-28a (+) متصل شد و سازه (pET-28a:PVS:CP) به باکتری *Escherichia coli* سویه BL21 منتقل شد. بهینه‌سازی بیان ژن با القا بوسیله IPTG در غلظت‌های ۰/۵، ۱ و ۲ mM به مدت ۳، ٤ و ٦ ساعت انجام گرفت و توسط SDS-PAGE و وسترن بلات تایید شد. بر اساس توالی نوکلئوتیدی، جدایه‌های ایرانی PVS به سه گروه تقسیم شدند که یک گروه با ۲۲ عضو به عنوان گروه غالب شناخته شد. بیان پروتئین نوترکیب با وزن ۳٤ کیلودالتون توسط وسترن تایید شد. القاء با غلظت ۱ میلی‌مولار IPTG و به مدت ٤ ساعت بهترین بیان را نشان داد. این نخستین گزارش بیان ژن پوشش پروتئینی ویروس PVS است که برای تهیه آنتی‌بادی این ویروس اهمیت دارد.

واژه‌های کلیدی

ویروس اس سیب‌زمینی،
بیان ژن،
پروتئین نوترکیب،
تجزیه فیلوژنتیکی،
PVS

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Introduction

Potato virus S (PVS) is one of the most important and widespread viruses infecting potatoes (Cox & Jones, 2010). Almost all varieties of potatoes are infected by PVS and infection rates up to 100% has also been reported in some cultivars (Hooker, 1983). Necrotic spots on the upper surface of leaves, vein banding, slight yellowing, leaf margin undulation and mottling, older plants wilt and plant stunting are symptoms of PVS (Hooker, 1983; Khurana, 1985). In mixed infections, PVS has synergistic effect with other plant viruses, such as *Potato virus X*, that can cause more damages (German, 2001). The virus belongs to the genus *Carlavirus* in the family *Betaflexiviridae* (King *et al.,* 2012). PVS has relatively flexible filamentous particles with approximate dimensions of 11-18 × 609-720 nm (Mathews, 1993). The virus genome is comprised of a single-stranded, positive sense RNA that contains 6 large Open Reading Frames (ORF) (Matousek *et al.,* 2005). The virus coat protein is translated from subgenomic RNA (ORF 5) and has 34 kDa molecular weight (Gramstat *et al*., 1990).

PVS has been classified in two strains namely ordinary strain (PVS^O) and Andean strain (PVS^A) on the basis of transmission by aphids and systemic and non-systemic infection in *Chenopodium* spp. (Foster & Mills, 1992). The PVS^A is transmitted by aphids in a non persistent manner, whereas the aphids do not transmit PVS^O. All detected isolates from Iran are related to PVS^O strain (Pourrahim *et al*., 2007; Salari *et al*., 2011).

The first detection of PVS in Iran is from Fars and Azerbaijan farms in 1974 based on the symptoms but further studies using ELISA and molecular tools were revealed the presence of virus in all parts of the country (Salari *et al.*, 2006; Pourrahim *et al*., 2007; Salari *et al*., 2011).

Virus detection is the critical step in the control of plant viruses and, in large numbers of samples specifically in certification schemes, it is still done by serological methods due to their robustness and low cost (Ling *et al*., 2000; Zimmermann *et al*., 1990). Expression of viral genes in *E. coli* has been an important strategy obtain large-scale recombinant proteins that can be used for production of virus-specific antibodies. One of the major advantages of recombinant protein technology is the high specificity antibody production with no cross-reaction to plant protein. The use of protein expression technology has been used to produce recombinant antibodies against some plant viruses (Sokhandan-Bashir *et al*., 2015; Lotfi *et al*., 2015; Lee & chang, 2008; Cerovska *et al*., 2012; Jain *et al*., 2005; Abou-Javad *et al*., 2004; Cerovska *et al*., 2003; Hourani & Abou-Javad, 2003; Korimbocus *et al*., 2002; Kumari *et al*., 2001).

The objectives of this study were to determine the dominant strain (s) of PVS in Iran and to express the PVS CP gene in *E. coli* that can be used as antigen for large scale production of PVS antibody.

Materials and Methods

Collection and serological detection of PVS

In order to detect PVS and identify it's dominant strain(s) in Iran, 176 leaf samples were collected from potato fields of Hamedan, Markazi, Kerman, Khorasan-razavi, Fars, Eastern Azarbaijan, Ardebil and Khuzestan provinces in 2015 and 2016. Samples were collected according to the symptoms of the virus and in some cases from symptomless plants. Most of the sampled plants had mosaic symptoms, systemic yellowing and leaf malformation. All samples were tested for PVS by double antibody sandwich enzyme-linked immunosorbant assay (DAS-ELISA) using virus specific polyclonal antisera purchased from the Agdia (USA) according to the manufacturer's instructions. The PVS-infected samples were identified by measuring the optical density of the microplate wells at 405 nm using ELISA reader (BIO-TEK, ELx808).

RNA extraction, cDNA synthesis and PCR amplification

Dominant isolate (s) of PVS was identified by genetic diversity studies between the Iranian isolates using reverse transcription polymerase chain reaction (RT-PCR) and amplification of partial PVS genome covering PVS coat protein (CP) gene. Total RNAs were extracted from the all ELISA-positive samples using RNA extraction kit (Vivantis, South Korea) according to manufacturer's instruction and subjected to cDNA synthesis using M-MuLV reverse transcriptase and random hexamer primer. A pair of primers, PVS-F (5´CAGTCCGGCTAGTCAATTGC3´) and PVS-R (5´CTCTGACTTTGCACCATGGG3´), were designed based on the PVS sequences retrieved from National Biotechnology Information Center, NCBI (accession numbers LN851193-4, Y15625, AJ863509, JX183954, FJ813513, AJ889246, JX183949). Recognizing protected region and primer designing were done using online ClustalW and Primer3 software, respectively. According to those accession numbers the primer pair supposed to amplify a 1317 base pair (bp) amplicon including PVS CP. Several isolates from different regions and provinces (according to the studied areas in previous studies) were selected for sequencing.

Sequencing and phylogeenetical analysis

Sequencing of RT-PCR fragments was performed directly by automated method Bioneer Inc (South Korea). The resulted sequences were aligned together with the corresponding regions of the previously reported isolates using GeneDoc (Nicholas & Nicholas, 1997). Phylogenetic analysis was done and Maximum likelihood phylogenetic trees of the CP region were constructed using MEGA5 software.

Cloning and construction of expression plasmid

Full length CP gene of the dominant PVS isolate lacking the start codon (ATG) was amplified from the cDNA of this

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isolate by a pair of newly-designed primers (pp-F: 5´AAGAGCTCCCGCCCAAACCGGAT3´ and pp-R: 5´TTAAGCTTTCATTGGTTGATCGCATT3´) possessing restriction sites *Sac*I and *Hin*dIII at the 5' and 3' positions of the fragment, respectively. These restriction sites (underlined) were engineered in these primers to facilitate cloning of CP into the expression vector. To do so, PCR was carried out in 50 µl PCR reaction mixture containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol each primers pp-F and pp-R, 4 µl cDNA and 1 unit *pfu*-DNA polymerase (Fermentas, Vilnius, Lithuania). The thermo cycle profile included 1 cycle of 94°C for 3 min; 35 cycles of 94°C for 30 sec, 62°C for 40 sec, 72°C for 90 sec and 1 cycle of 72°C for 5 min. The genomic fragment was extracted from the agarose gel using the gel extraction kit (Vivantis GF-1, South Korea). The amplified fragment was then inserted into the cloning vector pJET1/2.blunt (Thermo fisher Scientific, Lithuania), transformed into *E. coli* strain XL1-Blue (Chung *et al*., 1989) and cultured on Luria–Bertani (LB) medium containing ampicillin (100 µg/ml). The existence of recombinant plasmids was evaluated using colony PCR by PVS specific pp-F and pp-R primers. The recombinant plasmids were then extracted from colonies using the alkaline lysis method, were digested by *Sac*I + *Hin*dIII and finally evaluated on 1.5% agarose gel electrophoresis. The plasmids were further sequenced to confirm the lack of ATG codon and the presence of restriction sites.

The plasmid pJET:PVS:CP and expression vector pET-28a (Novagen, Germany) were double digested by *Sac*I/*Hin*dIII at 37°C for 12 hours and electrophoresed on agarose gel 1.5%. Then DNA fragments of PVS:CP and the linearized pET-28a vector were purified from agarose gel, ligated and transformed into *E. coli* XL1-Blue strain using heat-shock method (Chung *et al*., 1989). Transformants were selected on LB (tryptone 1%, yeast extract 0/5%, NaCl 1%) plates containing Kanamycin (50 µg/ml). Plasmid was extracted using the alkaline lysis method and subjected to *Sac*I/*Hin*dIII restriction analysis before sequencing. Sequencings were done with the T7 promoter and terminator primers (Bioneer, South Korea).

Expression and analysis of PVS CP

The expression vector carrying PVS:CP (pET-28a: PVS:CP) was transformed into *E. coli* strain BL21 and the transformants were inoculated on LB medium containing 50 µg/ml kanamycin and incubated overnight (ON) on a shaker incubator (37ºC, 200 rpm). The ON culture was diluted 50 times with fresh LB media containing kanamycin and shacked with 200 rpm at 37ºC to gain the optical density of 0.4-0.6 at 600 nm wave length. 1.5 ml volume of culture was sampled as non-induced bacteria for later analysis. Isopropyl-β-D-1-thiogalactopyranoside (IPTG) at concentrations of 0.5, 1 and 2mM were added to bacterial culture to induce the T7 promoter. Several volumes of 1.5 ml samples from the cultures were taken for analyses after 3, 4 and 6 h of the induction and centrifuged at 9000 g for 7-10 min in a Sigma 3-16PK rotor 3041 (Germany).

Western blotting

Proteins from the harvested cells were extracted with 160 µl of Laemmli buffer (125 mM Tris/HCl, pH 6.8, 2% sodium dodecyl sulfate, 9% glycerol, 0.7 M 2-mercaptoethanol, and 0.002% bromo phenol blue) and boiled for five minutes. Proteins of the supernatant and pellet fractions of the cell lysate were separated on two 12% Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (each sample 20-30 µl) (Laemmli, 1970) for 3 h at 100 V. One gel was stained with coomassie brilliant blue G-250 and followed by de-staining in buffer (H_2O 80%, methanol 10%, glacial acetic acid 10%). The unstained gel was blotted onto a nitrocellulose membrane (Amersham Hybound, UK) by a wet electro-transfer apparatus (Akhtarian, Tehran, Iran). The membrane was probed with 1:1000 dilution of commercial anti-PVS IgG (in PBS) (Agdia, USA) and incubated with goat anti-rabbit IgG-alkaline phosphatase conjugate. Then, 66 µl of Nitro Blue Tetrazolium (NBT) was added into 10 ml alkaline phosphatase buffer, before mixing with 32 µl of 5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) (Roche, Germany) to stain the membrane until the targeted protein bands reached the desired intensity. The reaction was stopped by washing the membrane in deionized water for several minutes, before the membrane was air-dried on a filter paper and photographed.

Results and Discussion

We analyzed 176 samples that taken from 8 provinces in Iran and 49 (27.8%) samples were positive in DAS-ELISA. The presence of PVS in ELISA-positive samples were approved by PVS-F/PVS-R primers in RT-PCR and amplified fragments of 1317 base pairs (bp) in all samples (Fig 1). Six samples were selected randomly for cloning and sequencing. The data of sequencing were submitted to BLASTN facility in the NCBI website (www.ncbi.nlm.nih.gov) to identify the best matching sequences recorded in GeneBank. The BLASTN revealed that all sequences belong to PVS. These sequences (accession numbers KY523839 to KY523844) along with other reported isolates from Iran were studied phylogenetically (Table 1).

We analyzed 83 isolates of PVS, which consisted of 41 isolates from Iran and 42 isolates from different parts of the world using Maximum likelihood method. There are two clear frequency distribution, accounting for PVS^O and PVS^A. Maximum likelihood analysis of the CP gene indicates that the all of the Iranian isolates belong to PVS^O strain and PVS^O isolates could be divided into 15 genotypes (Fig 2-B). The 41 Iranian isolates belong to O1 (9 isolates), O2 (2 isolates), O3 (4 isolates), O4 (1 isolates) O7 (2 isolates), O12 (4 isolates), O13 (5 isolates), O14 (1 isolates) and O15 (13 isolates) genotype (Table 2). This phylogenetic analysis shows that some degree of genotype/geographical region

specificity. O2, 3 and 4 genotypes absolutely contain Iranian isolates and O8 and 9 are predominantly found in Australia.

After determining the dominant strain and amplification of the complete PVS: CP without start codon, cloning was performed in cloning vector pJET 1.2/blunt and the sequencing result confirmed the successful attachment of fragment into the vector. Using double- digested with *Sac*I/*Hin*dIII the desired fragment was released from recombinant plasmid (pJET: PVS: CP) and the expression vector (pET-28a) was also linearized.

After ligation, nucleotide sequencing data confirmed in-phase insertion of the full length PVS:CP gene in the expression vector. pET-28a:PVS:CP was transformed into *E. coli* BL21 and protein expression was induced by IPTG. SDS-PAGE analysis of the total proteins harvested from the bacterial cells carrying pET-28a:PVS:CP and induced by IPTG in the range of 0.5–2 mM (final concentration) for 3, 4 and 6 hours showed a higher expression level of the expected protein of approximately 35 kDa in size corresponding to the PVS CP (Gramstat *et al*., 1990), whereas no CP band was observed with the non-induced sample (Fig 3).

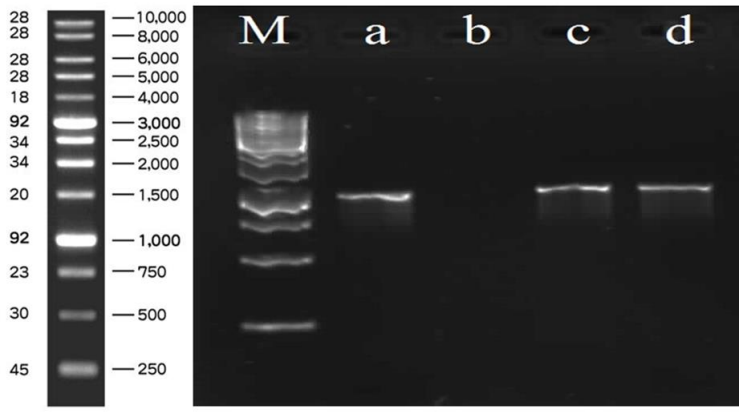

Figure 1. Electrophoresis of representative RT-PCR results from selected PVS isolates using PVS-F/PVS-R primers. Lanes: M: ladder 1Kbp; a, c and d: 1317 bp fragments from three isolates of PVS. b: negative control.

The molecular analysis of coat protein genes of the Iranian PVS isolates and their phylogenetic tree pattern classified the isolates into three main groups (A to C). However, the group A with 22 isolates is selected as the dominant group (Fig 2-A). Group C with 14 isolates and group B with 5 isolates were classified at the next levels according to the number of individuals. An isolate from Group A was selected as the representative of the dominant group. However, in order to be more reliable, the amino acid sequences of these isolates were also studied and the phylogenetic tree was drawn. The analysis and classification based on amino acid were similar to the results of nucleic acid analysis.

Table 1. Details of Iranian PVS isolates.

Isolate	Provinces/Region	Accession number	Isolate	Provinces/Region	Accession number
216-S	Kerman	KC853751	KHO.CH.3	Khorasan- Chenaran	HQ875141
KER.JI.6	Kerman- jiroft	HQ875137	Ara.2	Markazi- Ebrahimabad	KY523840
352-S	Kerman	KC853756	226-S	Khorasan shomali	KC853752
691-S	Esfahan	KC853763	490-S	Khorasan razavi	KC853758
652-S	Esfahan	KC853762	492-S	Khorasan razavi	KC853759
KER.SA.28	Kerman- Sardo	HQ875140	515-S	Khorasan razavi	KC853761
354-S	Hamedan	KC853757	KER.JI.4	Kerman- Jiroft	HQ875136
AZA.TA	Azarbaijan-sharghi-Tabriz	HQ875132	FAR.SI.14	Fars- Siakhan	HQ875134
Ham.3	Hamedan- Bahar	KY523841	KER.LA.15	Kerman- Lalezar	HQ875139
Ham.7	Hamedan	KY523844	KER.LA.12	Kerman- Lalezar	HQ875138
Ham.4	Hamedan	KY523842	Kho.chenaran	Khorasan- Chenaran	MF773985
ESF.FA.19	Esfahan- Fereydoonshahr	HQ875133	Kho.bojnord	Khorasan- Bojnord	MF773984
HAM.KA.20	Hamedan- Kabootarahang	HQ875135	Ker.soghan	Kerman- Soghan	MF773983
286-S	Kerman	KC853753	Ker.jiroft	Kerman- Jiroft	MF773982
KER.SA.21	Kerman- Saghan	HQ875142	Ker-anabarabad2	Kerman- Anbarabad	MF773981
314-S	Kerman	KC853754	Ker-anabarabad1	Kerman- Anbarabad	MF773980
315-S	Kerman	KC853755	Fars-Zarghan	Fars-Zarghan	MF773979
Dez.4	Khoozestan- Dezfool	KY523839	Fars-Shiraz	Fars-Shiraz	MF773978
Ara.6	Markazi- Arak	KY523844	Ag-9	Ardabil	MH159207
501-S	Khorasan razavi	KC853760	OS-11	Ardabil	MH159208
KHO.FA.2	Khorasan- Fariman	HQ856834			

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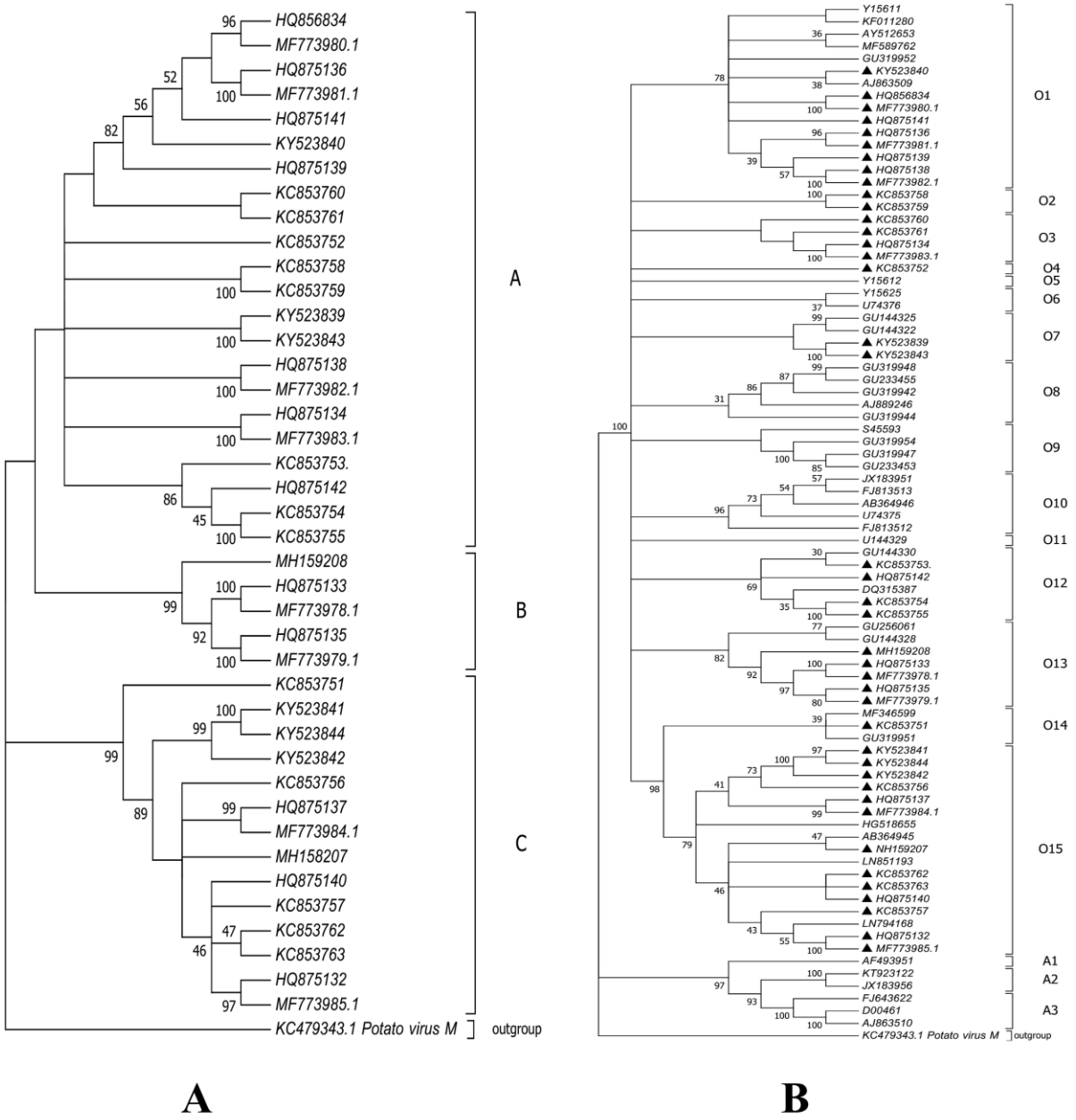

Figure 2. Phylogeny of: A) 41 Iranian isolates of PVS that was plotted based on complete gene of CP. The tree was constructed using the Maximum liklihood method and bootstrap analysis was applied using 100 replicates. Bootstrap values higher than 40 are indicated on nodes. PVM as an out-group was entered in this tree. B) 83 isolates of PVS (41 Iranian isolates and 42 isolates from world) that was plotted based on complete gene of CP. The tree was constructed using the Maximum liklihood method and bootstrap analysis was applied using 100 replicates. Bootstrap values higher than 30 are indicated on nodes. PVM as an out-group was entered in this tree.

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Table 2. Details of PVS genotypes.

Genotype group	country/Region	Accession number
O1	Czech Republic	Y15611
	China	KF011280
	China	AY512653
	Bangladesh	MF589762
	Netherlands	GU319952
	Germany	AJ863509
	Iran	KY523840
	Iran	HQ856834
	Iran	MF773980
	Iran	HQ875141
	Iran	HQ875136
	Iran	MF773981
	Iran	HQ875139
	Iran	HQ875138
	Iran	MF773982
O2	Iran	KC853758
	Iran	KC853759
O3	Iran	KC853760
	Iran	KC853761
	Iran	HQ875134
	Iran	MF773983
O4	Iran	KC853752
O5	Czech Republic	Y15612
O6	Czech Republic	Y15625
	South Korea	U74376
O7	United Kingdom	GU144325
	United Kingdom	GU144322
	Iran	KY523839
	Iran	KY523843
O8	Australia	GU319948
	Australia	GU233455
	Australia	GU319942
	China	AJ889246
	Australia	GU319944
O9	United Kingdom	S45593
	Australia	GU319954
	Australia	GU319947
	Australia	GU233453
O10	USA	JX183951
	USA	FJ813513
	Syria	AB364946
	South Korea	U74375
	USA	FJ813512
O11	United Kingdom	GU144329
O12	United Kingdom	GU144330
	Iran	KC853753
	Iran	HQ875142
	China	DQ315387
	Iran	KC853754
	Iran	KC853755
O13	India	GU256061
	United Kingdom	GU144328
	Iran	MH159208
	Iran	HQ875133
	Iran	MF773978
	Iran	HQ875135
	Iran	MF773979
O14	Slovakia	MF346599
	Netherlands	GU319951
	Iran	KC853751
O15	Hungary	HG518655
	Iran	KY523841
	Iran	KY523844
	Iran	KY523842
	Iran	KC853756
	Iran	HQ875137
	Iran	MF773984
	Syria	AB364945
	Iran	MH159207
	Ukraine	LN851193
	Iran	KC853762
	Iran	KC853763
	Iran	HQ875140
	Iran	KC853757
	Hungary	LN794168
	Iran	HQ875132
	Iran	MF773985
A1	Unknown	AF493951
A2	Brazil	KT923122
	Shile	JX183956
A3	India	FJ643622
	Peru	D00461
	Czech Republic	AJ863510

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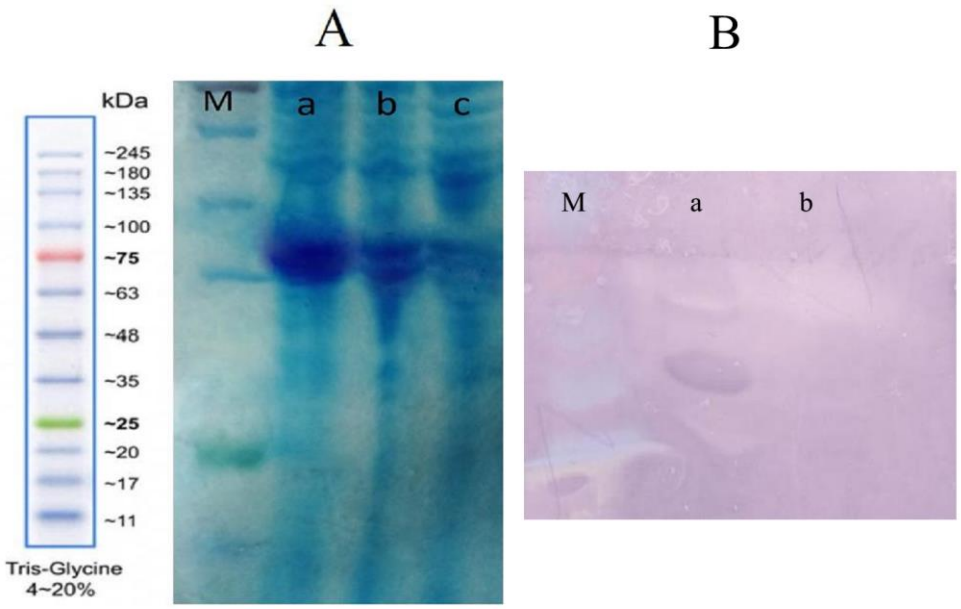

Figure 3. SDS-PAGE (A) and western blotting (B) of the expressed pET-28a:PVS:CP in *E. coli.* Lanes: M. Prestained protein marker; a. total protein from induced cells after 4 hours of inductions by 1mM IPTG; b. protein sample from non-induced cells; c. total protein from *E. coli* carrying pET-28a and lacking the PVS CP gene (A). Western blotting of PVS: CP on nitrocellulose membrane. Lanes: M. protein marker, a. expressed PVS:CP; b. protein sample from *E. coli* carrying pET-28a and lacking CP gene (B).

SDS-PAGE of the harvested proteins from different concentrations of IPTG in different induction periods revealed 1mM IPTG as the best concentration for the highest protein expression up to 4 hours induction (data not shown). However, beyond 4 h of induction, there was no increase in the expression level. The identity of the expressed protein was confirmed to be PVS CP by Western blotting, where a corresponding band was revealed on the nitrocellulose membrane.

In this study, two separate phylogenetic analysis, one for Iranian PVS isolates and one for isolates from different parts of the world were done. Compared to previous studies, more Iranian isolates (41 isolates) were used for phylogenetic analysis in this study that it helps to identifying dominant groups of PVS in the country (Fig 2-A), in addition helps to a more detailed phylogenetic comparison of Iranian isolates with isolates from other parts of the world (Fig 2-B). Identification of the dominant group of PVS in the country and the expression of its coat proteins of can help to produce a better and broader recombinant antibody.

According to the results, the PVS coat protein gene has a high genetic diversity, but despite this high diversity, the group with the highest number was considered as the dominant group in the country. In phylogenetic analysis of Iranian isolates with isolates from other parts of the world, Iranian isolates were divided into 9 groups of 15 identified groups and the two groups (O1 and O15) had the most Iranian isolates. This dominance of the two groups for Iranian isolates corresponds with Salari *et al*. (2011).

The study of nucleotide and amino acid sequences of the PVS CP gene, showed that all of the Iranian isolates belong to PVS^{O} strain. It also showed a high genetic diversity in this region that confirmed the results of similar previous investigations (Matousek *et al*., 2005; Pourrrahim *et al*., 2007; Salari *et al*., 2011). Salari *et al*. (2011) proposed that PVS^{O} isolates could be classified into 15 clades and the findings of our research are consistent with this. Expression systems, usually have advantages and limitations. The pET vector systems are of the most commonly used vectors in expressing recombinant proteins that have been used in gene expressions of various plant viruses (Sokhandan-Bashir *et al*., 2015; Koolivand *et al*., 2017; Rostami *et al*., 2014). Apart from the pET vector system, a number of other vector systems such as pGEX (Sokhandan-Bashir *et al*., 2015; Lotfi *et al*., 2015), pTrcHis (Lee & Chang, 2008) and pTBG (H) (Cerovska *et al*., 2012) have also been used for expression of foreign genes in *E. coli*. *Escherichia coli*, as a prokaryotic system has efficient generation time, fast high-density cultivation, high expression capacities and low cost and is known as a successful host for heterologous expression of foreign genes. Accordingly, the expression of CP genes from numerous plant viruses has been successfully done in *E. coli* (Abou-Javad *et al*., 2004; Chen *et al*., 2002; Fajardo *et al*., 2007; Plchova *et al*., 2011).

The expressed PVS CP was analyzed by SDS-PAGE and the presence of a band with molecular mass of approximately 34 kDa corresponding to that of PVS CP was observed. An

about 2 kDa difference with the PVS-CP mass was due to the fused Histidine tags.

The identity of the expressed protein was demonstrated to be PVS CP by western blotting. Although with the *E. coli* carrying pET-28a:PVS:CP (but not induced by IPTG), a weak reaction was obtained in western blotting (Fig 3), such background reactions are not unexpected considering the fact that a basal expression can occur either in the absence of IPTG (Gibbstown, 2007). Various IPTG concentrations and duration for expression of interested gene at a particular IPTG concentration are important factors in expression levels. These include IPTG at final concentration of 1 mM (Thomas & Baneyx, 1996) and 0.1 mM (Kumari *et al*., 2001) for the expression of *Prunus necrotic ring spot cirus* CP in *E. coli*, incubation at 37°C for three to four hours (Bragard *et al*., 2000; Liu *et al*., 2001), and at 16°C overnight has been reported (Kadkhodayan *et al*., 2000). In our study, we tested IPTG concentrations ranging from 0.5 to 2 mM and found that changes in this range does not heavily affect the expression level (data are not shown) and it is in accordance to another studies (Koolivand *et al*., 2017; Rostami *et al*., 2015). Also, we used induction periods of three to six hours and found that an induction by 1mM IPTG for four hours gives the best result.

This research showed that PVS CP open reading frame placed under the control of T7 promoter is efficiently expressed in *E. coli* strain BL21 and after induction of expression, the recombinant coat protein was confirmed by western blotting. This recombinant coat protein is under further efforts for production of virus-specific antibodies.

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Identification of dominant isolate of *Potato virus S* in Iran and heterologous expression of its coat protein

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Abstract

Gene expression in bacteria have already been used to study the protein of viruses and to produce specific antibodies. In this research, potato samples were collected from eight provinces of Iran and were tested for *Potato virus S*, as one of the most destructive viruses of potato fields, by DAS-ELISA and RT-PCR. Dominant isolate of PVS was selected according to coat protein (CP) gene sequences following phylogenetic analysis. Full length CP gene was amplified, cloned and sequenced, then was digested from pJET1.2/blunt vector, ligated into the expression vector pET-28a and the construct (pET-28a:PVS:CP) was transformed into *Escherichia coli* BL21 strain. Gene expression was optimized by induction with 0.5, 1 and 2 mM final concentrations of IPTG for 3, 4 and 6 h and was verified by SDS-PAGE and Western blotting. Based on the nucleotide analysis, the Iranian isolates of PVS were divided into three groups that one group with 22 members known as the dominant group. The expression of recombinant CP of about 34 kDa was proved by western blotting. Induction by 1 mM IPTG for 4 h proved to be the most efficient method of expression. This is the first report of the expression of PVS CP gene, which is important for the preparation of anti-PVS antibody.

Key words: *Potato virus S*, Gene expression, Recombinant protein, Phylogenetic analysis, PVS

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