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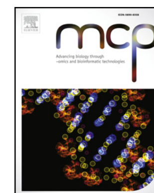
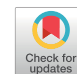


Comparing the efficiency of different *Escherichia coli* strains in producing recombinant capsid protein of porcine circovirus type 2



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ABSTRACT

The present study was aimed at comparing different *E. coli* strains in expressing the capsid protein of Porcine Circovirus 2 (PCV2). Full length capsid protein could be expressed only in Rosetta-gami 2 (DE3) pLysS strain using pET32b (+) vector. This confirmed that only those strains which possess tRNAs for rare codons can express the full length capsid protein. Purification of full length capsid protein could not be achieved even after several attempts using native and denaturing conditions. Subsequently, an attempt was made for expression of N-terminal truncated capsid protein using the same expression system. Truncated capsid protein was successfully expressed, purified and characterized by western blotting. The truncated capsid protein was also shown to be efficacious in testing serum samples using an optimized indirect ELISA, wherein a diagnostic sensitivity of 88.89% and specificity of 90.82% was obtained as compared to commercially available GreenSpring® porcine circovirus (PCV2) ELISA test kit. Thus, the expressed truncated capsid protein appears to be a promising diagnostic agent for PCV2. The comparative analysis suggests that cluster of arginine residues at N-terminal of capsid protein not only affects its expression in some *E. coli* strains but also its purification by Ni-NTA chromatography, when expressed as a histidine tagged fusion protein.

1. Introduction

Porcine circovirus type 2 (PCV2), a member of the *Circoviridae* family, is a single stranded DNA virus having circular genome [1]. They are found to be associated with a wide variety of disease syndromes in pigs, collectively called porcine circovirus associated diseases (PCVAD) or porcine circovirus diseases (PCVD) [2,3]. They are ubiquitous organisms and found in almost all parts of the world.

The genome size of PCV2 is only about 1767–1768 nucleotides in size and encodes 3 major ORFs [4]. ORF1 encodes for protein involved in replication of the virus [5] and ORF3 was shown to have a role in apoptosis in vitro and was also found to be associated with the pathogenicity of the virus in mice during an in vivo study [6]. ORF2 protein or capsid protein encoded by ORF2 is the only structural protein of the virus and determines the antigenicity of the virus [7]. This protein consists of several immunodominant epitopes and reacts strongly with serum of PCV2 infected animal [8,9]. Therefore, the capsid protein has been targeted by many workers for in-vitro expression, either for the development of vaccines or its use as a diagnostic agent [9–12].

Prokaryotic expression system is usually the most preferred expression system because it is simple and relatively inexpensive than eukaryotic systems. However, the expression of capsid protein in *E. coli* have had its fair share of difficulties. It is well documented that ORF2 of PCV2 contains a nuclear localization signal (NLS), which consists of multiple arginine residues encoded mostly by codons that are rarely used in *E. coli* [13]. These properties make the full length capsid protein of PCV2 a difficult protein to be expressed in prokaryotic cells. The NLS of the virus has a role in formation of stable virus like particles (VLPs) by the capsid protein [14]. Thus, it might be sometimes necessary to express the full length capsid protein, particularly when the ultimate aim is development of vaccines. Truncated capsid protein can serve the purpose when the ultimate aim is development of diagnostics. Since different *E. coli* strains differ in their properties from one another, which can have an effect on expression of heterologous proteins, the

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efforts of this study were directed towards the expression of capsid protein in different *E. coli* strains. Expression and purification of recombinant protein was confirmed using SDS-PAGE and western blotting. Western blot analysis using anti-PCV2 polyclonal serum and monoclonal anti-his antibody was done to confirm its specificity as the capsid protein was expressed as a fusion protein having histidine tag. Also, a truncated version of the same protein was expressed, purified and characterized using exactly the same system. An indirect ELISA was optimized and its efficiency to detect anti-PCV2 antibody was compared to a commercially available indirect ELISA kit. The outcome of this study will aid the future works in selecting the correct strain and method for recombinant capsid protein production, either for vaccine or diagnostic development.

2. Materials and methods

2.1. Strains and reagents

E. coli strains- BL21 (DE3) (cat. no. 69450–3, Novagen), BL21 (DE3) pLysS (cat. no. 69451–3, Novagen), Origami 2 (DE3) pLysS (cat. no. 71346–3, Novagen) and Rosetta-gami 2 (DE3) pLysS (cat. no. 71352–3, Novagen) were used in this study. Ni-NTA agarose was procured from Qiagen. Anti-His monoclonal antibody, goat anti-mice HRPO conjugate, rabbit anti-pig HRPO conjugate used in western blotting were purchased from Sigma. Commercial anti-PCV2 polyclonal serum used in western blotting was purchased from VMRD.

2.2. Preparation of recombinant constructs for expression

Total DNA was isolated from the suspected tissue sample using QIAamp DNA Mini Kit (Qiagen) and subjected to PCR using primers described by Larochelle [15]. Expression primers for approximately full length ORF2 (693 bp; designated ORF2) and truncated ORF2 genes (567 bp; designated PCAP42) were designed based on the PCV2 gene sequence (Gen Bank Accession No. KX009481). The descriptions of all primer pairs are given in Table 1. ORF2 gene was amplified after 35 cycles of PCR (95 °C, 1 min; 56 °C, 1 min; 72 °C, 1 min) and a final extension at 72 °C for 5 min. PCAP42, lacking the first N-terminal 126 nucleotides was amplified after 35 cycles of amplification (95 °C, 1 min; 62.4 °C, 1 min; 72 °C, 1 min) and a final extension at 72 °C for 5 min.

The PCR amplified products were purified by using GeneJET gel extraction and DNA cleanup micro Kit (Thermofisher) and cloned into pTZ57 R/T vector system using Thermo scientific InsTAclone PCR Cloning Kit according to the manufacturer's protocol. Sub-cloning of ORF2 was done in both pET28b (+) and pET32b (+) vectors (Merck), whereas sub-cloning of PCAP42 was done in pET32b (+) vector. Thus, three recombinant expression constructs were prepared, designated- pET28b/ORF2, pET32b/ORF2, and pET32b/PCAP42. Isolation and confirmation of positive pET28b/ORF2, pET32b/ORF2 plasmids in each step was done using RE double digestion and sequencing. Confirmation of positive pET32b/PCAP42 plasmids in each step was done using RE double digestion.

2.3. Transformation of different E. coli strains with recombinant plasmids

Expression constructs pET28b/ORF2 and pET32b/ORF2 were transformed in competent cells of *E. coli* strains- BL21 (DE3), BL21 (DE3) pLysS, Origami 2 (DE3) pLysS and Rosetta-gami 2 (DE3) pLysS. Expression plasmid pET32b/PCAP42 was transformed only in Rosetta-gami 2 (DE3) pLysS cells. Positive clones were selected using RE digestion.

2.4. Expression study of the recombinant construct in expression hosts

Strains pET28b/ORF2/BL21 were grown in Luria-Bertani (LB) medium containing 50 μg/ml kanamycin, whereas strains pET28b/ORF2/BL21 (DE3) pLysS, pET28b/ORF2/Origami 2 (DE3) pLysS & pET28b/ORF2/Rosetta-gami 2 (DE3) pLysS were grown in LB medium containing 50 μg/ml kanamycin and 50 μg/ml chloramphenicol. Similarly, LB medium with 50 μg/ml ampicillin was used for pET32b/ORF2/BL21 strains and LB medium with 50 μg/ml ampicillin along with 50 μg/ml chloramphenicol was used for pET32b/ORF2/BL21 (DE3) pLysS, pET32b/ORF2/Origami 2 (DE3) pLysS, pET32b/ORF2/Rosetta-gami 2 (DE3) pLysS & pET32b/PCAP42/Rosetta-gami 2 (DE3) pLysS strains. Cultures were incubated at 37 °C overnight with constant shaking @200 rpm. Next day, cells were diluted 1:100 and allowed to grow at 37 °C to an optical density of 0.6 at 600 nm. Different isopropyl β-D thiogalactopyranoside (IPTG) concentrations i.e. 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM were used to determine optimum concentration for induction of protein expression, after which cells were harvested by centrifugation. Similarly, different incubation period for harvesting the cells post IPTG induction were used to determine optimum time for peak protein expression. In each experiment, an un-induced culture was kept as negative control. Expression of the desired proteins was confirmed using SDS-PAGE and Western blot analysis. The collected cell pellets were also subjected to solubility analysis by SDS-PAGE using standard protocol [16].

2.5. Western blot analysis

Expressed proteins were resolved in 15% polyacrylamide and transferred to Nitrocellulose membrane (Merck Millipore) sandwiched between extra thick filter paper pad (BioRad) and equilibrated with transfer buffer (20 mM Tris–HCl, 190 mM glycine, 20% methanol, pH 8.3) using trans-blot apparatus (BioRad). The membrane was then blocked with blocking buffer (5% skim milk powder in PBS- 0.1% tween-20) followed by incubation with 1:5000 diluted monoclonal anti-his antibody and 1:5000 diluted polyclonal goat anti-mouse antibody, respectively. Signal development was done using 3,3′-diamino benzidine (DAB) substrate solution (0.05% DAB, 0.015% H_2O_2 in sodium acetate buffer).

2.6. Purification of recombinant ORF2 and PCAP42 protein

The expressed proteins bearing a histidine tag was purified under denaturing conditions using Nickel-nitrilotriacetic acid (Ni-NTA) column (Qiagen) as per manufacturer's instructions. Briefly describing, the pelleted bacterial cells were resuspended in binding buffer (pH 8.0)

Table 1
Details of primers for ORF2 gene used for confirmation of PCV2 and its subsequent expression. Restriction sites in the expression primers are shown in bold letters.

Sr. No.	Gene	Primer	Sequence (5′ to 3′)	Reference
1	ORF2	CF8	TAGGTTAGGGCTGTGGCCTT	Larochelle et al. [15]
		CR8	CCGCACCTTCGGATATACTG	Larochelle et al. [15]
2	ORF2	PCAP-F	**AAATCGATGGATCCG**ATGACGTATCCAAGGAGG	This study
		PCAP-R	**GTCGACCTGCAGGGTACC**AAGTGGGGGGTCTTTAAG	This study
3	PCAP42	PCAP-42 F	**GCATGGATCCAGATATC**ATGGGCATCTTCAACACCCGCC	This study
		PCAP-R	**GTCGACCTGCAGGGTACC**AAGTGGGGGGTCTTTAAG	This study

(8 M urea, 100 mM sodium dihydrogen phosphate, 10 mM tris-Cl). Different combinations of protease inhibitors were added to the binding buffer such as Phenylmethylsulfonyl fluoride (PMSF), protease inhibitor cocktail (Sigma). The cell lysates were sonicated ten times at amplitude of 20 (20 s pulse and 20 s pause) at 4 °C, followed by centrifugation at 14000*g* for 30 min at 4 °C to pellet the cells debris. 5 ml binding buffer was added to equilibrate Ni-NTA columns and centrifuged at 2000g for 2 min. The supernatant containing solubilized protein was added into equilibrated 50%. Ni-NTA agarose slurry at the rate of 1 ml of the 50% Ni-NTA slurry to 4 ml lysate and the tubes were incubated at 4 °C for 1 h with occasional shaking. The lysate-resin mixtures were carefully loaded into empty columns, allowed to settle and washed 10 times with 5 ml of wash buffer (pH 6.3) (8 M urea, 100 mM sodium dihydrogen phosphate, 10 mM tris-Cl). The proteins bound to resins were eluted several times by passing it through different volumes of elution buffer (pH 4.5) (8 M urea, 100 mM sodium dihydrogen phosphate, 10 mM tris-Cl). Exactly similar protocols were followed for purification of both ORF2 and PCAP42 protein. Urea was removed from different fractions using 30 kDaAmicon Ultra 15 mL Centrifugal Filters (Merck Millipore). The concentration of purified protein was determined by Bradford assay using reagents purchased from Sigma, in 96 well plate using manufacturer's protocol before storing it at −20 °C until further use. Eluted proteins were analyzed by SDS-PAGE using standard protocol and western blotting as described in the previous section. Western blotting was performed using both monoclonal anti-his antibody and commercial anti-PCV2 polyclonal serum. Anti-PCV2 polyclonal serum was used at a dilution of 1:250 whereas rabbit anti-pig HRPO conjugate was used at a dilution of 1:4000.

2.7. Indirect ELISA using truncated capsid protein as antigen (PCAP42 ELISA)

At first, a checkerboard titration was performed to determine optimum working dilution of antigen, test serum and conjugate for PCAP42 ELISA. Then, 107 sera samples (n = 107) were tested by GreenSpring® porcine circovirus (PCV2) ELISA Test kit (#LSY-30007 Porcine Circovirus (PCV2) ELISA test kit, Shenzhen Lvshiyuan Biotechnology) as per manufacturer's instructions. The same samples were then analyzed using PCAP42 ELISA. Briefly describing the protocol, individual wells in the ELISA plate were coated with 300 ng of purified PCAP42 protein diluted in coating buffer (Carbonate-Bicarbonate buffer, Ph 9.6) and incubated for 1 h at 37 °C under constant shaking. After washing 3 times with wash buffer (Phosphate buffer saline with 0.05% tween-20), 300 μl blocking buffer (Phosphate buffer saline, 0.1% tween-20 and 5% Skim milk powder) was added and incubated for 1 h at 37 °C. Positive control, negative control and test sera at 1:50 dilution in dilution buffer (Phosphate buffer saline, 0.1% tween-20 and 5% Skim milk powder) were added in duplicates after washing all the wells 3 times with wash buffer. After further incubation for 1 h at 37 °C under constant shaking and washing 3 times with wash buffer, rabbit anti-pig IgG-HRPO conjugate was added at a dilution of 1:5000 in dilution buffer and the plate was incubated again under similar conditions. Following washing 4 times with wash buffer, 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution was added to each well and after development for 10 min, the reaction was stopped with 1 M sulphuric acid. OD_{450} of all the wells were measured and S/P ratio (Sample/Positive ratio) for each sample was calculated using the formula:

$$S/P\ ratio = (Sample\ OD_{450} - NC)/(PC - NC)$$

where,

PC- OD_{450} of positive control
NC- OD_{450} of negative control

Receiver-operating characteristic (ROC) curve data was used to establish the negative-positive cut-off value and evaluation of diagnostic sensitivity and specificity of the ELISA relative to commercial ELISA used in this study.

3. Results

3.1. Preparation of recombinant constructs for expression

PCR conducted on the isolated genomic material revealed a band of expected molecular weight i.e. around 263 bp, detected in 1% agarose gel electrophoresis, which helped in the selection of PCV2 positive DNA for further amplification. Using the positive genomic material as template in subsequent PCR reactions, ORF2 gene was amplified as a 726 bp product, which comprised of gene specific sequence (693 bp) and nucleotides of restriction enzymes sites present in the forward and reverse primers. PCAP42 gene was amplified as 602 bp product.

The purified PCR products were cloned into pTZ57 R/T vector using TA-cloning strategy. This was followed by sub-cloning of ORF2 in pET28b (+) and pET32b (+) expression vectors and PCAP42 in pET32b (+) vector. The recombinant plasmids isolated in each step were shown to be positive by BamHI/SalI digestion at 37 °C. RE digestion of pET32b/ORF2 and pET32b/PCAP42 released an insert of 716 bp and 596 bp, respectively (Fig. 1A and 1B). In addition, sequencing also confirmed the positive pET28b/ORF2 and pET32b/ORF2 constructs (data not included).

3.2. Expression analysis of recombinant proteins by SDS-PAGE

Different *E. coli* strains were transformed with recombinant pET28b/ORF2 plasmid and pET32b/ORF2 plasmid. It was observed that the full length capsid protein was expressed only in Rosetta-gami 2 (DE3) pLysS cells harbouring pET32b/ORF2 plasmid. Solubility analysis using SDS-PAGE revealed that the protein was expressed in the form of inclusion bodies (data not shown). A protein band of approximately 47 kDa size was seen in lanes loaded with induced cell lysate of pET32b-ORF2-Rosetta-gami 2 (DE3) pLysS clones (Fig. 2A), whereas no such band was seen in un-induced cell lysate or lanes loaded with cell lysates of other clones. No *E. coli* strain transformed with pET28b/ORF2 could express the desirable protein. Maximum protein expression was seen at 5 h post induction with 1 mM IPTG. PCAP42 protein was expressed as inclusion bodies by Rosetta-gami 2 (DE3) pLysS cells harbouring pET32b/PCAP42 plasmid (data not shown). Expression was confirmed by SDS-PAGE analysis where a band of 42 kDa was observed in lanes loaded with culture lysate induced by 1 mM IPTG whereas the same band was absent in lanes loaded with un-induced lysate (Fig. 2B). Maximum truncated capsid protein expression was seen at 7 h post induction with 1 mM IPTG.

3.3. Purification of recombinant proteins

The expressed proteins were subjected to Ni-NTA chromatography purification strategy, but the full length capsid protein couldn't be purified even after several attempts. No bands were observed in SDS-PAGE and Western blot analysis of the purified fractions. Similar results were seen even after the use of different protease inhibitors. PCAP42 protein was successfully purified under denaturing conditions using Ni-NTA affinity chromatography in different fractions. The identity of the purified fractions was confirmed by SDS-PAGE analysis where a band of 42 kDa was seen in the loaded lanes (Fig. 3).

3.4. Western blot analysis of expressed and purified proteins

To further confirm the expression and specificity of the ORF2 protein, induced culture lysates of recombinant clones were analyzed by western blotting using monoclonal anti-his antibody. A brown band of

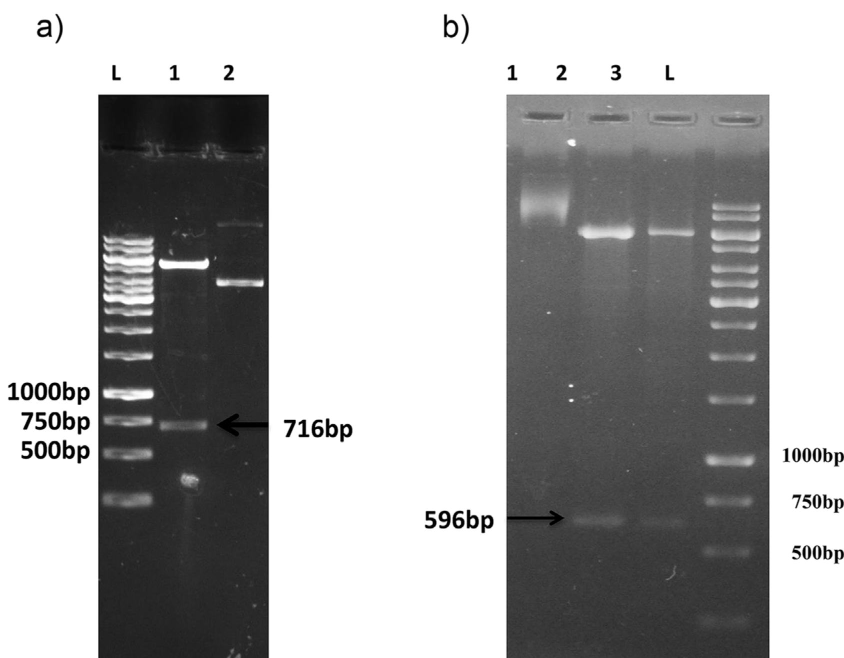


Fig. 1. Confirmation of cloning and sub-cloning of ORF2 gene. **(A)** RE digestion of pET32b/ORF2 plasmid, where lanes L, 1 and 2 were loaded with DNA ladder (1 kb+), BamHI/SalI digested plasmid and undigested plasmid respectively. **(B)** RE digestion of pET32b/PCAP42 plasmid, where undigested plasmid was loaded in lane 1, two BamHI/SalI digested plasmids were loaded in lane 2&3 and DNA ladder (1 kb+) was loaded in lane L.

similar size as seen in SDS-PAGE along with some streaking developed only in induced culture lysate due to reactivity of anti-his monoclonal antibody to the expressed full length fusion protein having histidine tag, whereas no band was observed in un-induced cell lysate (Fig. 4). The band was thick as large amount of samples were loaded in each well, whereas the streaking was probably due to reactivity of the antibody to *E. coli* proteins having poly-histidine residues. Similar analysis of PCAP42 protein using monoclonal anti-his antibody and anti-PCV2 polyclonal serum produced a band of 42 kDa in lanes loaded with induced culture lysate and purified protein elutes (Fig. 5A &B).

3.5. Indirect ELISA based on PCAP42 as antigen

Based on the results obtained by checkerboard titration, antigen concentration of 300 ng/well, serum dilution of 1:50 and conjugate dilution of 1:5000 were used for testing of serum samples. For blocking, serum dilution and conjugate dilution, 5% skimmed milk powder in 1X Phosphate buffer saline-tween-20 was used. Testing of 107 sera samples by GreenSpring® porcine circovirus (PCV2) ELISA Test kit revealed that 9 samples were positive and 98 samples were negative for PCV2 antibodies. Comparing the results obtained using PCAP42 ELISA to the results of GreenSpring® porcine circovirus (PCV2) ELISA Test kit using Graph pad prism software, the AUC indicated that the PCAP42 ELISA test was on average 92.63% accurate. The 95% confidence intervals (CI) of the AUC for the ELISA ranged from 87.51 to 97.75% and the significance level (Area = 0.5) was $P < 0.0001$. The receiver-

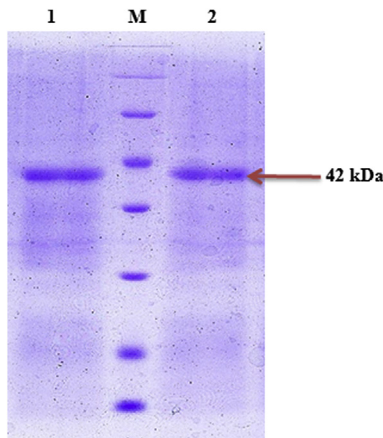


Fig. 3. SDS-PAGE analysis of purified PCAP42, where lanes 1 and 2 were loaded with different purified fractions and lane M was loaded with a protein marker.

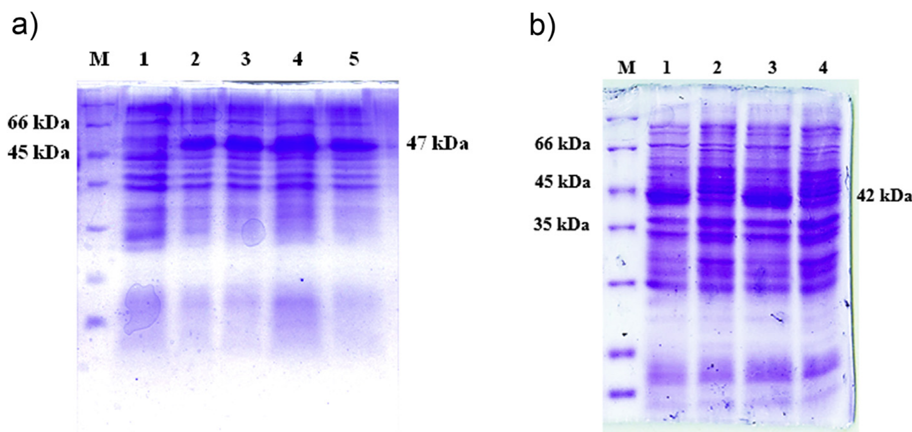


Fig. 2. (A) SDS-PAGE analysis of full length capsid protein expression in Rosetta-gami 2 (DE3) pLysS cells. Lane M was loaded with protein marker and lane 1 with un-induced cell lysate. Lanes 2, 3, 4 and 5 were loaded with cell lysates induced with 0.5 mM, 1.0 mM, 1.5 mM and 2.0 mM IPTG respectively.
(B) SDS-PAGE analysis of PCAP42 expression in Rosetta-gami 2 (DE3) pLysS cells. Lane M was loaded with protein marker, lane 1 & 2 with induced and un-induced cell lysate of one pET32b/PCAP42 clone. Lanes 3 & 4 were loaded with induced and un-induced cell lysates of a different pET32b/PCAP42 clone.

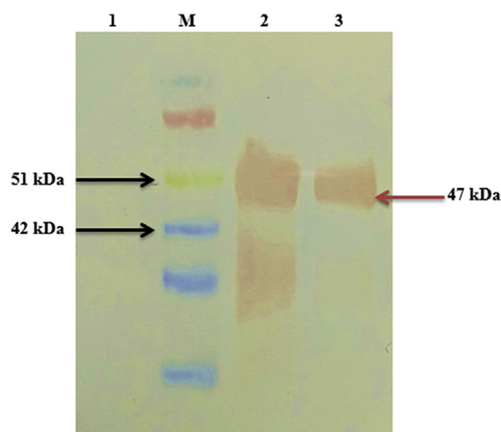


Fig. 4. Western blot analysis of full length capsid protein expression in Rosetta-gami 2 (DE3) pLysS cells using monoclonal anti-his antibody. Lane 1 was loaded with un-induced cell lysate. Lanes 2 and 3 were loaded with cell lysates from different clones induced with 1 mM IPTG. Lane M was loaded with pre-stained protein marker.

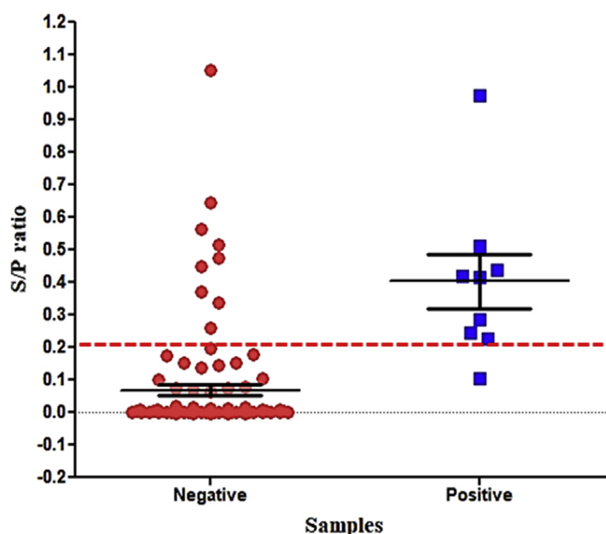


Fig. 6. A scatter plot showing distribution of tested samples by PCAP42 ELISA, when cut-off was fixed as 0.21 using ROC analysis.

operating characteristic (ROC) analysis showed that the optimal cut-off point was an S/P ratio of 0.21, corresponding to a sensitivity of 88.88% and specificity of 90.81% (Fig. 6).

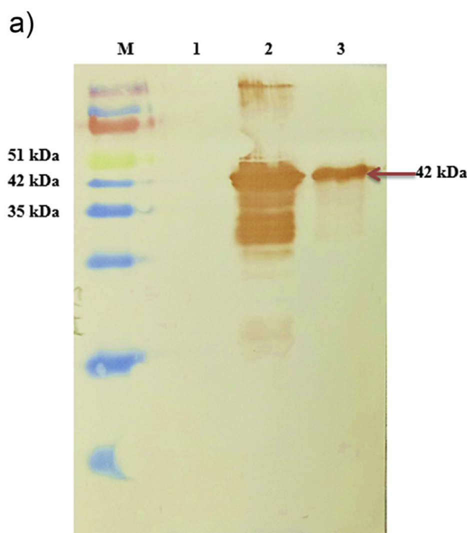
4. Discussion

ORF2 or capsid protein being the sole structural protein of PCV2 has been the centre of attention among researchers. On the other hand, prokaryotic expression system has its own popularity owing to its relative simplicity. So we were fixated on expressing the capsid protein in *E. coli* cells. Since it has been a well-known fact for quite some time now that it is difficult to express the full length capsid protein of PCV2 in prokaryotic system, we chose two expression vectors and four expression hosts as a part of our strategy to express this protein.

At first, the amplified ORF2 gene was cloned and sub-cloned into pET28b (+) expression vector. None of the four *E. coli* strains transformed with recombinant pET28b/ORF2 clone expressed the capsid protein, in contrast to the results obtained by Chen et al. [11]. So, ORF2 gene was again sub-cloned into pET32b (+) expression vector and the recombinant pET32b/ORF2 clones could express the desirable protein. The probable reason for this observation is the presence of a 109 amino acids long thioredoxin tag along with other tags present in pET32b (+) vector, which is absent in pET28b (+) vector. Attaching a highly translated native gene as a fusion on the N-terminal end of the heterologous target protein is an approach to improving yield [17,18].

However, of the four *E. coli* strains used, the desirable protein was expressed only in Rosetta-gami 2 (DE3) pLysS cells. Rosetta-gami 2 host strains have the properties of both Origami 2 and Rosetta 2, which includes allowing for enhanced disulphide bond formation and expression of heterologous proteins encoded by codons which are rarely used by *E. coli*. tRNAs for seven rare codons, AUA, AGG, AGA, CUA, CCC, GGA, and CGG are supplied by the chloramphenicol-resistant plasmid, pRARE2, carried by these cells. The presence of multiple arginine residues in the NLS of PCV2 encoded by rare codons requires these tRNAs to be expressed in *E. coli* cells. So, their absence in other cell strains resulted in our observation.

ORF2 protein was expressed as a fusion protein of approximately 47 kDa size as confirmed by SDS-PAGE and Western blot analysis. The expressed protein couldn't be purified or degraded rapidly upon purification after multiple attempts using Ni-NTA chromatography, even with the use of different protease inhibitors. A Similar observation was also reported by Chen et al. [11]. This suggests that the arginine clusters in capsid protein not only affects its expression but also has an additional effect on its purification using Ni-NTA chromatography. To test this hypothesis, exactly the same protocol was followed to express



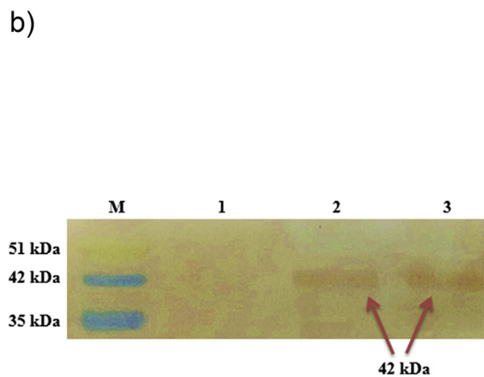


Fig. 5. (A) Western blot analysis of PCAP42 expression in Rosetta-gami 2 (DE3) pLysS cells and purified PCAP42 using monoclonal anti-his antibody, where Lane M was loaded with protein marker, lane 1 with un-induced cell lysate, lane 2 with induced cell lysate and lane 3 with purified PCAP42.
(B) Western blot analysis of purified PCAP42 protein using polyclonal anti-PCV2 serum, where lanes 1, 2 and 3 were loaded with different purified fractions of PCAP42 and Lane M was loaded with protein marker.

and purify a truncated version of the protein lacking few N-terminal amino acids. The truncated protein was successfully expressed, purified and characterized using SDS-PAGE and western blotting, in agreement with our hypothesis. Thus it can be assumed that the presence of multiple arginine residues encoded by rare codons in NLS of the virus somehow leads to the instability of the recombinant protein or makes it more susceptible to degradation. However more work on this aspect or other proteins with similar structure needs to be done to delineate the mechanism by which it occurs.

A total of 107 serum samples were screened by both commercial ELISA kit and PCAP42-ELISA developed in this study. ROC curve was used for determining the optimal cut–off of PCAP42 ELISA. This method has been used frequently by many to assess diagnostic accuracy of a test, to determine the optimal cut-off value of a test and to compare diagnostic accuracy of several tests [19–21]. Based on ROC curve data, the cut-off value fixed was 0.21 and the relative sensitivity and specificity obtained were 88.89% and 90.82%, respectively. However, only 9 out of the 107 samples were shown to be positive by the commercial kit. Only 1 sample out of 9 positive samples was found negative in PCAP42-ELISA, but the sensitivity dropped by around 12%, which does not indicate the true picture. Nevertheless, PCAP42 expressed in this study reacted specifically to anti-PCV2 serum, as evinced by western blotting and ELISA.

5. Conclusion

Full length ORF2 protein of PCV2 is a difficult protein to be expressed. Only the correct combination of expression vector having highly translated native gene and expression hosts having abundance of tRNAs for rare codons can express this protein. Even after expression, if this protein is expressed as a histidine tagged protein, it is highly unstable owing to the presence of multiple arginine residues which hinders the purification process. So, one should preferably express the full length capsid protein without the use of poly-histidine tags. It is better to express the truncated version of the protein without the NLS region, if the desired goal is development of diagnostics. The results generated from this work will benefit the future studies directed at development of recombinant capsid protein based diagnostics and vaccines against PCV2.

CRediT authorship contribution statement

Vishal Rai: Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Vikramaditya Upmanyu:** Conceptualization, Methodology, Resources. **Gulam Mohd:** Investigation, Formal analysis. **Ravi Kumar:** Investigation, Formal analysis. **Sanganagouda Koppad:** Investigation. **Aleema Ansari:** Investigation. **Durlav Prasad Bora:** Resources. **Awadh Bihari Pandey:** Validation. **Pronab Dhar:** Validation. **Ashok Kumar Tiwari:** Funding acquisition, Supervision.

Declaration of competing interest

None.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcp.2020.101556.

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