Recombinant Coat Protein of Banana Bract Mosaic Virus as a Potential Antigen for Serological Detection of the Virus

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Authors’ contributions
This work was carried out in collaboration among all authors. Author DD designed the study, performed the investigation and validated, wrote the protocol and wrote the first draft of the manuscript. Authors VL, PS and HSS provided the resources and supervised the study and edited the manuscript. Author PMN managed the literature searches. Author KAC provided resources and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT
Banana bract mosaic disease caused by Banana bract mosaic potyvirus (BBrMV) is reported to instigate heavy loss in banana and plantain across Asia. Almost all the cultivars of banana succumb to the disease resulting in malformed bunches weighing less than half of normal ones. In the current study the coat protein (CP) gene segment present at the 3' terminal region of the viral genome amplified by RT-PCR was cloned into expression vectors, pRSET-C and pGEX-4T-2 to use it for raising polyclonal antiserum which in turn will aid in developing assays to detect the virus. Recombinant BBrMV CP (rCP) in pRSET-C when expressed was insoluble whereas, it was in the
soluble fraction when expressed from pGEX-4T-2. The GST-fusion protein was purified by GSH sepharose affinity column chromatography and western blot analysis was performed using anti GST antibodies. 360 µg/ml of protein was purified from 1 l of culture. The GST tag was cleaved from the purified protein by incubation with thrombin at 25°C overnight. The rCP was characterized using ultracentrifugation, fluorescence spectroscopy and electron microscopy. The tagless monomer failed to assemble to virus like particles (VLPs) in vitro which was substantiated by fluorescence spectroscopy. This study will be first step towards deciphering structure and functions of Banana bract mosaic virus coat protein.

Keywords: Banana bract mosaic virus; recombinant coat protein; affinity column chromatography; virus like particles.

1. INTRODUCTION

Banana is the most consumed and produced fruit in India. The remunerative production of banana is challenged by viral diseases viz., Banana bunchy top disease (BBTD), Banana bract mosaic disease (BBrMD). Infectious chlorosis and Banana streak disease (BSD) [1]. Banana bract mosaic disease also called as “Kokkan” caused by Banana bract mosaic virus (BBrMV) is cosmopolitan and accounts for 40-70% economic loss annually in Asian countries [2-7]. Recently, occurrence of the disease has been reported in different parts of the world in crops like cardamom, ginger and Heliconia sp apart from banana [8-10]. It was first reported in 1979 on an island Mindanao in Philippines [11].

BBrMV a potyvirus, is a 9.7 Kb single stranded RNA virus encapsidated in 725 nm long flexuous rod [12-14]. The genome encodes for a polyprotein which is proteolytically processed into capsid protein and 10 other non-structural proteins by its own proteases [3,15-16]. The 34 kDa coat protein is encoded at the 3’ end of the genome.

BBrMV infection is observed in all the cultivars and loss incurred is huge irrespective of the variety. Large longitudinal pink coloured streaks, formed on the pseudostem and petiole, due to the disease at early stages of plant growth is often confused with that of the varietal characters of most of the cultivated varieties in India [17]. The conspicuous symptom is observed as dark and light purple patches on the bract giving a mosaic appearance during the reproductive stage of the crop, which causes irreparable loss and difficulty in crop management. Less common symptoms like ‘traveller’s palm’ like leaf arrangement [18] and necrotic streaks on fruits, leaves, pseudostem and midribs [17] have also been reported. Transmission of the virus is primarily by planting materials. However, association with insect vectors viz., Pentalonia nigronervosa, Aphis gossypii, Rhopalosiphum maydis [5] and A. craccivora [19] is seldom observed.

The period of latency of the virus to showcase symptoms in the infected plants is not only a threat to the domestic market especially where it is vegetatively propagated but also to international germplasm exchange [20]. The tissue culture planting materials that look prima facie healthy, especially have to be indexed for the presence of this virus [21]. Hence, early detection is the key for controlling the disease along with providing virus indexed high quality planting materials to the farmers until a resistant variety is identified and used for breeding [1]. Timely diagnosis will also ensure an arrest to the spread of the virus through infected planting material and facilitate uninterrupted international germplasm exchange.

Enzyme linked immunosorbent assay (ELISA) is widely preferred for virus indexing as it is economical and reliable [13,22-23]. However, the sensitivity and specificity of the method depends upon the quality of antiserum used for detection [24] and evidently the quality of antiserum is determined by the quality of the antigen used to raise it. Partial purification of the virus from infected planting materials yields virions along with many protein contaminants of plant origin. Moreover, higher concentration of phenols in banana results in low quality of the protein. Purified recombinant coat protein (rCP) overexpressed in a bacterial host is the answer to the constraints of obtaining high quality pure antigen. However, the success of the technique is restrained to the solubility of the protein thus obtained. This study is aimed at expressing and purifying soluble recombinant coat protein in Escherichia coli so that immunodiagnostic techniques could be developed and optimized for sensitive and specific detection of the virus.
2. MATERIALS AND METHODS

Media and bacteriological reagents were primarily from Hi-Media, Mumbai, India. Other chemicals and reagents, were purchased from Sigma-Aldrich. The oligonucleotides were synthesised from Eurofins, Bangalore (India). DNA modifying enzymes and restriction enzymes were obtained from Thermo Fisher and New England Biolabs. The bacterial strains used in the study are E. coli DH5α, E. coli strains BL21(DE3) and Rosetta (DE3) containing pLysS.

2.1 Sample Collection

A roving survey was conducted in Northern, Central and Southern zones of Kerala. Young leaves of banana showing BBTD symptoms were collected from different varieties grown in various location of Kerala, India. Infected suckers from some mother plants were maintained in insect free net house, Banana Research Station, Kannara, Kerala.

2.2 Preliminary Assay by Direct Antigen Coating-Enzyme Linked Immunosorbent Assay (DAC-ELISA)

Twenty five samples were collected and subjected to preliminary assay by DAC-ELISA with slight variation in the standard protocol [25]. Antigen was isolated in carbonate buffer (pH 9.2) and coated on polystyrene 96 welled ELISA plate (Tarsons Pvt. Ltd.) for 1 h at 37°C. After washing the excess antigen using PBS-T (PBS of pH 7.4 containing 0.1 % v/v Tween-20), blocking buffer (5% skim milk powder in PBS-T) was added and kept for 45 min at 37°C. Post washing the excess blocking buffer with PBS-T, 1:200 v/v of commercially available potyviral primary antibody (Agdia Inc.) in PBS-TPO buffer (PBS-T with 2% Poly vinyl pyrrolidone and 0.2% bovine serum albumin) was added to the wells and incubated overnight at 4°C. The excess primary antibody was washed and anti-rabbit IgG conjugated with alkaline phosphatase enzyme (Sigma Aldrich USA, 1:10000 v/v) was added to the wells and incubated for 2 h at 37°C. Substrate solution (1000 ppm of para-nitro phenyl phosphate disodium salt in diethanolamine, pH 9.8) was added to the wells after washing off excess secondary antibody and incubated for 30 min in dark at room temperature. The reaction was stopped by adding 3 M NaOH solution and the absorbance was recorded at 405 nm in ELISA reader. Two healthy samples as negative control and two positive samples as positive control were included in the assay. The test samples that showed higher than double the A405 value of negative controls were taken as positive samples.

2.3 RNA Extraction, RT-PCR and Sequencing

Total RNA was isolated from 12 representative samples that gave positive results in ELISA using RNeasy Plant MiniKit (Qiagen, USA) according to manufacturer’s instructions. The cDNA synthesis was carried out using Oligo (dT)18 primer in RevertAid First strand cDNA synthesis kit (ThermoFisher Scientific). The cDNA was used as template for Polymerase Chain Reaction (PCR). The coat protein gene of 12 isolates were amplified using forward primer and reverse primer (Table 1) [26] under the following conditions: 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 42°C for 1 min, 72°C for 2 min with a final extension for 10 min at 72°C. The Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) products were separated by 1.2% Agarose gel and purified PCR products (PCR purification kit, ThermoFisher Scientific) were sequenced by Sanger dyeoxy sequencing method.

2.4 Cloning and Overexpression of BBrMV Coat Protein

The purified PCR product was gel eluted using GeneJET gel extraction kit (Thermo Fisher Scientific) as per manufacturer’s protocol. It was cloned to TA cloning vector, pGEM-T easy (Promega). Confirmation of clone was carried out by restriction digestion with EcoRI (ThermoFisher Scientific) and sequencing. The coat protein gene of BBrMV was amplified from cDNA clone in pGEM-T easy vector using primers so designed as to incorporate Nhe1 and BamH1 restriction sites (underlined) to the 5’ and 3’ of the gene respectively for cloning in to pRSET-C and pGEX-4T-2 expression vectors (Table 1).

The coat protein (CP) gene was amplified using high fidelity Phusion (Pfu) polymerase (New England Biolabs, Inc., MA) under the following conditions: 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 30 s, annealing at 55°C for 1 min, extension at 72°C for 45 s and final extension at 72°C for 10 min. The amplified PCR product was electrophorized on 1.2% Agarose
2.5 Western Blot Analysis

The expression of recombinant coat protein was confirmed using western blot analysis. The lysates of equal concentration were loaded on to 12% gel and SDS-PAGE was performed. The proteins bands were electrophoretically blotted on to nitrocellulose membrane (Hybond-C, Amersham) [27]. The unoccupied sites of the blot were blocked using blocking buffer (5% skim milk powder in PBS) for 1 h at room temperature to eliminate non-specific binding of the antibodies followed by washing with PBS-T. The blot was then incubated in primary antibody (1:5000 v/v anti His monoclonal antibody and 1:5000 v/v anti-GST polyclonal antibody) overnight at 4°C. The blot was washed thrice with PBS-T and twice with PBS to remove unbound antibodies. In order to detect his-tagged rCP, goat anti-mouse monoclonal IgG conjugated with Horseradish peroxide (HRP) was used as secondary antibody. Goat anti-rabbit polyclonal IgG-HRP conjugate was used as secondary antibody to detect GST fusion protein. After repeated washing with PBS-T, Immobilon chemiluminescent HRP substrate (Millipore) was used for detection as per manufacturer’s protocol.

2.6 Purification of Recombinant Coat Protein

To purify rCP, the pGEX-4T-2 clone was overexpressed in 1 l YT broth by induction with 0.5 mM IPTG and incubated at 16°C for 14 h with constant shaking at 100 rpm. Cells were harvested by centrifugation for 20 min at 8000 rpm 4°C and resuspended in 50 mM Tris (pH 8.0) containing 150 mM NaCl, 5% glycerol and 1% Triton-X 100. The cells were lysed by sonication for 10 min on ice bath giving 3 s interval between 6 s pulse of 35% amplitude. The debris was separated from crude lysate by centrifugation at 12000 rpm 4°C for 20 min. One ml calibrated GSH sepharose beads were allowed to bind to the soluble fraction for 5 h at 4°C in an end to end rotor. Column was packed with protein resin complex and washed twice to remove the unbound proteins with 10X bed volume of wash buffer (50 mM Tris, pH 8.0 and 150 mM NaCl). The recombinant fusion protein was eluted with buffer containing 50 mM Tris, pH 8.0, 200 mM NaCl and 30 mM glutathione. The eluted fractions were pooled and dialysed against 50mM Tris, pH 8.0 containing 150 mM NaCl and 10% glycerol to remove glutathione.

The purified fusion protein (0.5 mg) was incubated with 2.5 U thrombin (Millipore) to remove GST tag. To separate rCP from GST protein, the digested protein was allowed to bind to GSH sepharose beads and tag less recombinant protein alone was collected. On-column digestion using 10 U/ml bead volume of thrombin to obtain the tag less protein was attempted at 25°C for 8, 12 and 14 h to compare the best method for removal of the tag. The purity of the protein was checked in SDS -PAGE and quantified by Bradford’s assay.
2.7 Assessment of Self-Assembly of Tag Less Protein

The purified tag less recombinant BBrMV CP was subjected to ultracentrifugation at 26,000 rpm for 3 h at 4°C (Beckman coulter, MLS 50 rotor). The supernatant and pellet were separated using SDS-PAGE. The pellet was dissolved in minimum volume of Tris NaCl buffer (50 mM Tris, pH 8.0 and 150 mM NaCl). Linear sucrose gradient (10-40%) were poured to ultracentrifuge tubes and equilibrated overnight at 4°C. The pellet suspension was layered on the linear sucrose gradient and centrifuged at 26,000 rpm for 3 h at 4°C. Fractions of 250 µl were collected and analysed on SDS-PAGE. The peak fractions were pooled and subjected to ultracentrifugation at 26,000 rpm for 3 h at 4°C. The ultra pellet obtained was resuspended in Tris NaCl buffer.

2.8 Transmission Electron Microscopy

In order to assess the pattern of self assembly, 0.1 mg/ml of ultracentrifuged rCP was absorbed on formvar coated copper grids (SPI supplies, USA) for 2 mins. After removing the excess sample it was negatively stained with 2% uranyl acetate (w/v) for 1 min. The air dried grids were viewed under Tecnai F30 Transmission Electron Microscope (ThermoFisher, USA) at 1,60,000X magnification.

2.9 Fluorescence Spectrometry of rCP

The intrinsic fluorescence was measured using TECAN LS55 Luminescence Spectrometer (Perkin-Elmer). Spectra were recorded at protein concentrations of 0.1-0.5 µg in buffer containing 50 mM Tris, pH 8.0 and 150 mM NaCl. Blank was set with the same buffer without recombinant protein. The excitation wavelength was 280 nm and the emission was scanned between 300-400 nm.

3. RESULTS AND DISCUSSION

3.1 In silico Analysis

The sequence of BBrMV CP from pGEM-T recombinant clone was subjected to BLAST against the nucleotide database of NCBI. The 5’ and 3’ end of the gene was determined. As the potyviral genome is translated to a polyprotein which is later cleaved to proteins of various size and function by its own proteases [28], the coat protein does not start with amino acid methionine. Expasy Translate tool was used to obtain the translated amino acid sequence of the protein and it was observed that protein starts with a serine residue. The FoldIndex analysis [29] revealed that 32 amino acid residues at the N terminal and 47 at C terminal are disordered (Fig 1A). These regions have many protein binding domains involved in interaction with the helper component proteinase (HC-Pro) for aphid transmissibility and facilitation of cell to cell movement [30]. The N terminal region varies from 30-67 amino acids in potyvirids, whereas length of C terminal differs by one or two amino acids [15]. The disordered N and C terminal regions of Pepper vein banding potyvirus (PVBV) have shown to be essential for the initiation of viral assembly by forming a ring like intermediate consisting of eight subunits [31]. The core region of the coat protein especially the arginine residues is RNA binding motif which is highly conserved across the potyviridae family (Fig. 1B). [32] The same was reported by Shukla and co-workers in 1988 and hypothesised that these reacts with viral nucleic acid [15]. However, N-terminal was variable and largely consists of the virus specific epitopes which contribute to virus specific antibodies [33].

In a study by Rodoni and co-workers [4], high degree of divergence of BBrMV Indian isolates was highlighted and hypothesised that, this phenomenon may be due to the prolonged presence of the virus in this area. Further investigation on genetic diversity and recombination events in coat protein of BBrMV Indian isolates corroborated the findings of aforementioned authors [14]. It was also concluded that the high variability of BBrMV population and occurrence of new recombinants may pose a challenge in breaking pathogen derived resistance. BBrMD in India was first observed since 1966 in Kerala [3] and has spread over various neighbouring states in five decades. Evidently the genetic diversity of Kerala isolates has to be considered while designing methods for detection of the virus. The amino acid sequence variability is observed more at the N-terminal region of the coat protein of isolates collected from various parts of Kerala (result not shown here).

3.2 Overexpression of Recombinant BBrMV CP Fusion Protein

The pRSET-C clone harbouring BBrMV CP gene when overexpressed in E. coli BL21 (DE3) pLysS
and Rosetta pLysS bacterial systems, showed a doublet band at 34 kDa (Fig. 2). Formerly, few other potyviral coat proteins have also been reported to migrate as doublet in SDS-PAGE due to partial degradation of polypeptide by host or microbial proteolytic enzymes [34-36]. Nevertheless, turnip mosaic virus coat protein was reported to show three bands in SDS-PAGE profile and a single band was observed in the case of sugarcane mosaic virus coat protein [37-39].

Maximum expression of BBrMV rCP was found at 16 ºC in YT broth, however maximum cell growth was observed in TB. Most of the protein expressed in both the cell lines was in the insoluble fraction (Fig. 2). Western blot analysis against anti-histidine monoclonal antibody revealed that the protein expressed at 34 kDa was indeed BBrMV CP (Fig. 3). The N-terminal histidine tag was detected by the anti-his antibody, producing single band instead of a doublet. Ni-NTA purification was attempted,

**Fig. 1. In silico analysis of translated BBrMV CP**

A) Predicted ordered (Green) and disordered (Red) regions in BBrMV CP by FoldIndex. B) Amino acid sequence with ordered (green) and disordered (red) regions as predicted by FoldIndex. The blue boxes indicate the highly conserved regions across Potyviridae family

**Fig. 2. SDS-PAGE for checking overexpression of BBrMV CP**

Lane 1: Protein molecular mass marker, Lane 2-7: overexpression of BBrMV CP/pRSET-C in BL21 (DE3) pLysS, Lane 8-13: overexpression of BBrMV CP/pRSET-C in Rosetta pLysS, Lane 2 and 8: soluble (supernatant) fractions of induced BBrMV CP in pRSET-C in LB, Lane 3 and 9: insoluble (pellet) fractions of induced BBrMV CP in pRSET-C in LB, Lane 4 and 10: soluble fractions of induced BBrMV CP in pRSET-C in TB, Lane 5 and 11: insoluble fractions of induced BBrMV CP in pRSET-C in TB, Lane 6 and 12: soluble fractions of induced BBrMV CP in pRSET-C in YT, Lane 7 and 13: insoluble fractions of induced BBrMV CP in pRSET-C in YT. The doublet at 35 kDa in the insoluble fraction indicated by red arrow.
which were also confirmed by western blot of the protein were observed in the SDS profile of the culture. However, when the purified protein was subjected to thrombin cleavage, multiple bands of the protein were observed in the SDS profile which were also confirmed by western blot analysis using anti-GST antibody (Fig. 6). The surface exposed N and C terminal of potyvirus CP is prone to proteolytic cleavage during purification or storage by proteases of microbial origin depending upon the host in which they are purified [15, 36, 40]. Nevertheless, GST tag also contributes to degradation due to unknown reasons. The multiple bands of the purified recombinant BBrMV CP could also be due to this proteolytic cleavage. Even though proteolysis of the tag less BBrMV CP was observed, an on-column thrombin digestion at 25°C for 8 h proved to be better than in-solution digestion and a band corresponding to 34 kDa was observed on the SDS-PAGE (Fig. 7).

3.3 Purification of Soluble Recombinant BBrMV CP

Non-specific high molecular weight proteins were eluted along with rCP when overexpressed in BL21pLysS harbouring recombinant pRSET-C/BBrMV CP. Therefore, to obtain a pure recombinant CP, BBrMV CP cloned into pGEX-4T-2 vector was used for purification as described in methods section. The recombinant coat protein (rCP) was purified by GSH Sepharose affinity column chromatography to 90% homogeneity and was highly soluble (Fig. 5). The purified rCP was also detected and confirmed using anti GST polyclonal antibody. 360 µg/ml of pure protein was obtained from 1 l culture. However, when the purified protein was subjected to thrombin cleavage, multiple bands of the protein were observed in the SDS profile which were also confirmed by western blot analysis using anti-GST antibody (Fig. 6). The earlier attempt to purify BBrMV capsid protein was conducted by Rodoni and co-workers in 1999 [4]. They cloned the CP in pProEX-1 vector and expressed in E. coli M15 cells to obtain a fusion protein of ~40 kDa which was used for developing diagnostics. Other potyviruses like PVBV coat protein and sugarcane streak mosaic virus (SCSMV) coat protein cloned into pRSET-C and pRSET-A vectors respectively were overexpressed in E. coli system and the soluble protein was purified using Ni-NTA affinity column chromatography [24, 41]. Sugarcane mosaic potyvirus coat protein was overexpressed and purified as fusion protein with MalE maltose binding protein (MBP) which was later cleaved by protease factor Xa [42]. Apparently, the solubility of the recombinant protein expressed, varies with expression vector and host used which is of paramount importance to obtain high yield of purified recombinant coat protein.
Assessment of BBrMV CP Self-Assembly through Ultracentrifugation

The BBrMV CP purified using GSH sepharose column chromatography was subjected to sucrose density gradient ultracentrifugation. Most of the recombinant protein was observed in 10% sucrose density gradient fractions on SDS PAGE profile. The electron micrograph resembled disc shaped intermediate of the viral like protein during the initial stage of assembly (Fig. 8).
The potyviral coat protein assembles as stacked rings after binding to the RNA at the origin of assembly and grows towards 5' and 3' end [31]. The assembly of viral like particles (VLP's) takes place in vitro in the presence or absence of RNA [43]. In vitro, the surface exposed N and C terminal of PVBV CP subunits interacts with each other in a head to tail manner to form octameric rings forming helical aggregates of varying length with N and C residues exposed. It was well established experimentally that truncated CP subunits cannot form VLPs [31]. Proteolysis of recombinant BBrMV CP post purification was suspected as multiple bands were observed in the SDS-PAGE profile. The failure of purified CP to assemble to VLPs may be attributed to partial proteolysis of C terminal residues (as N terminal GST tag was detected in western blot analysis) of few subunits.

Hitherto, Johnson grass mosaic virus CP has been expressed in E. coli, yeast, insect, and mammalian systems [44-46] and formation of filamentous VLPs of heterogenous length were testified in all the systems. PVBV capsid protein cloned to pRSET-C vector when overexpressed

Fig. 6. Western-blot analysis of purified recombinant fusion protein
Lane 1: Protein molecular mass marker marked on the blot post Ponceau’s staining, Lane 2: BBrMV CP fusion protein, Lane 3: Thrombin treated fusion protein. All the samples were run on 12% SDS-PAGE and western blot analysis was carried out using anti-GST polyclonal antibody

Fig. 7. SDS-PAGE (12 %) of thrombin digestion of rCP
Lane 1: Protein molecular mass marker, On-column thrombin digestion of fusion protein (BBrMV CP-GST) incubated with thrombin for different time periods; Lane 2: 8h, Lane 3: 12h, Lane 4: 14h
and purified, most of the protein was present in the soluble fraction. Also, PVBV CP could self-assemble to form VLPs in sucrose density gradient [41,47]. It was shown that the presence of extra amino acids in the N terminal (either due to cloning strategy or chimeric VLP) did not hamper the assembly [48]. However, BBrMV fusion protein with GST tag did not assemble into VLPs (data not shown) possibly because the tag is a huge peptide of 25 kDa. In the present study, BBrMV-CP cloned into pRSET-C was highly insoluble hence could not be analysed for self-assembly. The immunochemical screening of 296 octapeptide corresponding to amino acid sequences of the entire coat protein of Johnson grass mosaic potyvirus by Shukla and co-workers demonstrated the immunodominance of N-terminal when intact virions are used as an immunogen [49]. Thus, VLPs formed in vitro by self-assembly of the coat protein subunits would undoubtedly be best candidate for raising antiserum than monomeric CP.

3.5 Assessment of Tertiary Structure of Recombinant BBrMV CP through Intrinsic Fluorescence

The intrinsic fluorescence spectrum of untagged BBrMV CP measured in the 300 to 400 nm region had maximum (λmax) at ~344 nm. A globular protein in its native state typically exhibits intrinsic fluorescence maximum at 330 nm. The shift in fluorescence intensity of BBrMV CP denotes that the protein is folded with partial disordered region (Fig. 9).

![Image of TEM](image.png)

**Fig. 8.** Transmission electron micrograph of the purified recombinant BBrMV CP

TEM was performed after ultracentrifugation of purified tag less BBrMV CP to check self-assembly at 1,60,000x magnification. Red arrow corresponds to ring like structure formed during initiation of assembly.

![Image of Fluorescence Spectrum](image.png)

**Fig. 9.** Fluorescence spectrum of rBBrMV CP after removing GST tag

The excitation wavelength was set at 280 nm and the emission spectra was recorded from 300 - 400 nm.
This substantiates the FoldIndex analysis which depicted the N and C terminal of BBrMV CP to be disordered. It is also evident that a few of the phenylalanine, tryptophan and tyrosine residues, responsible for fluorescence are present at the surface exposed N and C terminal (Fig. 1B). Similar result was inferred from fluorescence spectrum of bean common mosaic virus coat protein which depicted λmax at 345 nm [50]. The fluorescence spectrum of BBrMV CP also indicated that the protein could be a monomer. The result validates the electron micrograph observed post self-assembly wherein, the protein was suspected as mostly monomeric with few octameric ring structures and not VLPs. The intact virion and coat protein of another potyvirus, Potato virus A exhibited a fluorescence spectrum with two maxima at 314 nm and ~330 nm suggesting both the virions and coat protein have similar structure, apparently CP could have assembled into VLPs [51,52]. The fluorescence intensity of TMV CP, was lower by two times the intensity of PVA CP [52] and is comparable to that of BBrMV CP.

4. CONCLUSION

The fusion protein pGEX-BBrMV CP overexpressed in BL21 (DE3) pLysS is highly soluble and eluted without any non-specific proteins. The rCP was characterized by western blotting, ultracentrifugation, electron microscopy and fluorescence spectroscopy. Further, biophysical and physicochemical characterizations like size exclusion chromatography, dynamic light scattering, differential static light scattering, circular dichroism and analytical ultracentrifugation of the protein in future could give insights into the secondary structure of the protein. In contrast to the cumbersome procedures for partial purification of virions from infected banana samples, highly pure recombinant coat protein of BBrMV can be obtained easily in large quantity by increasing the culture volume. Consequently, repeated immunization of animals would be possible to obtain anti-BBrMV polyclonal antibody with high titre value. The antiserum in turn will help in developing extremely specific and sensitive immunodetection of this RNA virus in field as well as tissue culture samples. Apart from its use in timely detection, the recombinant protein can also aid in comprehensive structural analysis which will pave the way to unravel the multifunctional properties of the coat protein and structure-function correlation as reported in few other potyviruses. In the way forward sustainable management strategies against viruses belonging to this family can be formulated using the results thus obtained.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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