Indonesian Journal of Biotechnology

VOLUME 24(1), 2019, 57–64 | RESEARCH ARTICLE

Expression and purification of recombinant coat protein of sugarcane mosaic virus from Indonesian isolate as an antigen for antibody production

Natalia Tri Astuti[1], Nurmalasari Darsono[2], Suvia Widyaningrum[3], Widhi Dyah Sawitri[4], Sri Puji Astuti Wahyuningsih[2], and Win Darmanto[2,*]

[1]Biotechnology Laboratory, PT. Perkebunan Nusantara XI, Jalan Merak 1, Surabaya 60175, Indonesia
[2]Biology Department, Faculty of Science and Technology, Airlangga University, C Campus, Jalan Mulyorejo, Surabaya 60115, Indonesia
[3]Post Graduate Program for Biotechnology, University of Jember, Jalan Kalimantan No. 37, Jember 68121, Indonesia
[4]Agronomy Department, Faculty of Agriculture, Universitas Gadjah Mada, Jalan Flora, Bulaksumur, Yogyakarta 55281, Indonesia
[*]Corresponding author: windarmanto@fst.unair.ac.id

SUBMITTED 1 May 2019 **REVISED** 10 June 2019 **ACCEPTED** 27 June 2019

ABSTRACT Sugarcane mosaic virus (SCMV, genus *Potyvirus*, family *Potyviridae*) is a prominent pathogen of sugarcane (*Saccharum* sp. hybrids). It can cause losses in susceptible varieties, in crops as well as sugar production, economically. Although it has been studied in major sugar-producing countries, research on the definement of SCMV from Indonesian isolates based on molecular studies has been very limited. This study aimed to obtain a proper recombinant antigen emanating from the coat protein of SCMV from an Indonesian isolate in order to produce polyclonal antibodies that can be used in immunodiagnosis assays in a subsequent study. A gene-encoding coat protein of SCMV (CP-SCMV) was amplified using RT-PCR and cloned into vector pJET1.2. The cDNA was inserted into the 6X His-tag expression plasmid of pET28a(+) and over-expressed in *Escherichia coli* BL21(DE3) to produce a recombinant protein. The highest expression was found in 0.1M IPTG induction media for 5 h at 37°C. SDS-PAGE analysis clarified that the recombinant CP-SCMV remained as an insoluble fraction. Purification was carried out by the affinity Ni-NTA resin, followed by electroelution to obtain a highly purified protein. To meet the quality requirements of a proper antigen, the highly purified protein was concentrated. A molecular weight of the rCP-SCMV (approximately 40 kDa) was clearly observed by 10% SDS-PAGE at the concentration of 16.184 mg/mL.

KEYWORDS antigen; cDNA; recombinant coat protein; sugarcane mosaic virus (SCMV)

1. Introduction

Sugarcane mosaic virus (SCMV) has been found to be a major viral pathogen in sugarcane-growing countries worldwide, causing a negative financial impact due to crop yield losses. In a recent study, researchers recorded a decline in biomass of around 10–32%, and in sugar yield, up to 10% was caused by mosaic disease (Anwar 2005). Mosaic disease affects the growing of sugarcane. The symptoms of the disease include discoloration of the leaves, in which some areas show a shade of light green or yellow due to chlorosis, and may be accompanied by varying degrees of necrosis.

The causal particle of sugarcane mosaic is related with SCMV, sorghum mosaic virus (SrMV), and sugarcane streak mosaic virus (SCSMV) (Xu et al. 2008). The first study on mosaic disease caused by SCSMV in Indonesia was reported by Damayanti and Putra (2011). Nevertheless, research on the defining of SCMV is very limited in Indonesia. According to Shukla et al. (1992), SCMV belongs to family *Potyviridae*, genus *Potyvirus*. The virions are flexible and rod-shaped, about 750 nm in length and have a genome of positive-sense RNA of around 10 kb long with a viral-encoded protein (VPg) covalently attached to the 5' terminus. There is a single, long open reading frame encoding a polyprotein. It is post-translationally processed into the individual gene and produced by viral proteases (Shukla et al. 1992).

Detection of SCMV in sugarcane plants has been performed molecularly through the reverse transcription (RT)-PCR technique. At present, molecular detection is still the most accurate method. In general, molecular methods of RT-PCR are not suitable for routine detection with a large number of samples. According to Fajardo et al. (2007), the usage of molecular methods for a large quantity of samples is not recommended because of high costs and lack of practicality. Therefore, a detection method that is more suitable to assessing a large number of samples is a serological approach using the enzyme-linked immunosorbent assay (ELISA). Early detection of the causal agent of mosaic disease is very important since it can be followed by preventing widespread viral infections. Efforts to pre-

vent the spread of the virus are also supported by the use of pathogen-resistant varieties and virus-free planting materials, as well as also destroying infected material in the sugarcane field or nursery.

The serological approach using immunoassays and their various versions, which uses antibodies as the main component, has been used universally for the detection and diagnosis of plant viruses, including SCMV (Khalil et al. 2007). Preparation of the conventional polyclonal antibody has limitations such as purity of the virion and low viral titer in the infected plant tissue (Fajardo et al. 2007). To overcome this problem, a serological method using polyclonal antibodies prepared against the recombinant coat protein is commonly used for immunodiagnostics. Several productions of polyclonal antibodies against the recombinant protein have been reported, such as the antibody against the Pelargonium zonate spot virus coat protein (Gulati-Sakhuja et al. 2009), cucumber mosaic virus coat protein (Khan et al. 2012), and alfalfa mosaic virus (Khatabi et al. 2012). Therefore, the method that utilizes bacterial expression systems for the production of coat proteins from plant viruses to be used as immunogens for the generation of antibodies is now a common method (Gulati-Sakhuja et al. 2009).

The expression and purification of recombinant proteins improves significantly with the additional fusion tag to facilitate the purification, solubility, and stability of the protein product. Conformational changes in virus epitopes may arise during purification, as monitored in grapevine virus B and partial degradation of antigens due to proteolysis during the storage of sugar beets, which can lead to low immunogenicity of the antiserum and subsequently affects serological detection of these viruses (Abdel-Salam et al. 2014), the proper antigen must be a highly purified protein with almost zero contaminant (≤ 1%). A purification technique using electroelution avoids contamination by other proteins that could appear during other protein purification techniques such as chromatography (Saraswat et al. 2013; Vázquez-Iglesias et al. 2017). The purified expressed plant viral protein as a recombinant fusion protein has been used as an antigen for raising virus-specific antibodies for immunodiagnosis. This study aimed to obtain recombinant antigens from the coat protein (CP)-SCMV as a proper antigen for polyclonal antibodies production to be used for immunodiagnosis assays.

2. Materials and methods

2.1. Amplification of Indonesian SCMV isolate and cloning of CP-SCMV gene into expression vector

An SCMV isolate of Indonesian origin was obtained from an infected sugarcane leaf in the field, in the Jember region, East Java Province, Indonesia (Addy et al. 2017). Virus isolation was conducted by sectioning sugarcane leaves showing symptoms of mosaic disease using scissors or a knife. The sugarcane leaf samples were then processed in the laboratory and used as RNA sources. Total RNA isolation was performed using an RNA prep kit according to the manufacturer's instructions (Tiangen, Taiwan), followed by storage in a deep freezer (80°C) for later use. The RNA was subsequently used as a template for cDNA synthesis using a first strand cDNA synthesis kit according to the manufacturer's instructions (Roche, Switzerland). The cDNA was then used as a template to amplify the full-length CP-SCMV in PCR using the forward primer 5'-CTCCCTGGGTATTTAGAGG-3' and reverse primer 5'-TTCCAGGAGACTAGTGGTG-3' to produce an amplicon of 1000 bp in length. PCR conditions were 95°C for 2 min, followed by 40 cycles of 98°C for 30 s, 56°C for 20 s, and 72°C for 1 min, with a final extension at 72°C for 5 min.

The PCR product was analyzed on 1% agarose gel in TAE buffer. The amplicon with the correct size was excised from the agarose gel using a sharp, disposable blade, put into an Eppendorf tube, and continued with purification using a DNA purification kit according to the manufacturer's manual (Promega, United States). The purified amplicon of full-length CP-SCMV was then directly cloned (ligated) into pJET1.2 using a TOP Fast plasmid cloning kit (Thermo Fisher Scientific, United States). The ligation product was then transformed into *E. coli* DH5α competent cells (GeneMark) using the heat shock method (Sambrook and Russell 2001). The inserted fragment was confirmed by growing colonies on the LB medium containing 50 ppm ampicillin. Several single colonies were picked from the LB medium using ose, and put into an Eppendorf tube containing the cocktail for PCR analysis.

The PCR program for the colony PCR was the same as the previous program for the cloning procedure. The results were visualized using electrophoresis on 1% agarose gel. The positive clones of full-length CP-SCMV were grown on LB liquid media containing ampicillin 50 ppm for overnight. Plasmid isolation was carried out using a plasmid miniprep kit according to the manufacturer's manual (Genomic DNA purification kit from Promega, United States). The plasmid was sent to 1st BASE, Malaysia, for sequencing analysis. Subsequently, the sequence data were compared with other SCMV sequences stored in GenBank using the Basic Local Alignment Search Tools nucleotide (BLASTn) program. The homology of the nucleotide and amino acid sequences were analyzed using Bioedit version 7.2.5.

For cloning to the expression vector, the CP-SCMV was PCR-amplified using the forward primer FL; 5'-GCGGATCCGTCGATGCAGGTGCTCAAGG-3' (BamHI enzyme site was underlined) and reverse primer RL; 5'-GTGCTCGAGCAGAGAGTGCAT-3' (XhoI enzyme site was underlined). The amplicon (± 925 base-pair) and expression vector pET-28a(+) were cut with restriction enzymes, BamHI and XhoI (Thermo Fisher Scientific, United States) using the double-digest method. The total volume used in the double-digest reaction was 10 µL, which consisted of buffer Xho1 and BamH1 1 µL, Xho1 and BamH1 enzymes 0.5 µL, RNAase 1 µL, ddH2O 2.5 µL, and plasmid as the template of 5 µL. All of the components were mixed slowly, then incubation was car-

ried out at 37°C overnight. The fragment gene was directly cloned into the expression vector pET-28a(+), and then transformed into competent *E. coli* cells BL21 (DE3) (Geneaid, Taiwan) using the heat shock method (Sambrook and Russell 2001), and ligated using T4 DNA Ligase (New England BioLabs, United States) following the manufacturer's instructions. Positive clones were determined using PCR analysis with a T7 promoter and terminator primers. One of the positive clones were reconfirmed again with PCR amplification using the conserved region primer set with restriction enzyme sites and the PCR program, as aforementioned.

2.2. Expression of rCP-SCMV and purification by Ni-NTA resin

Bacterial cultures were grown in 2x YT medium containing 50 mg/mL kanamycin, and were shaken at 150 rpm at a temperature of 37°C. Production of the recombinant protein was induced by the addition of IPTG at 0.1, 0.5, and 1 mM concentrations for 3 and 5 h at 37°C after optical density of the bacterial cultures reached 0.8. The bacterial cultures were harvested by centrifugation at 12,000 rpm at 4°C for 10 min. Pellet cells were used for extraction to obtain the recombinant protein. The pellets were homogenized with buffer NPI-10 (2 mL/gram) with the addition of 100 µg/mL lysozyme and then sonicated five times (30 s each time). In the next step, the soluble and insoluble fractions were separated by centrifugation at 12,000 rpm at 4°C for 10 min. The NPI-10 buffer was added into the pellet (insoluble fraction) and centrifuged again at 12,000 rpm at 4°C for 20 min. The supernatant was removed and the pellet was resuspended with DNPI-10 buffer and sonicated again for 3 min in order to solubilize the recombinant protein. The cell suspension was then centrifuged at 12,000 rpm at 20°C for 20 min. The supernatant was used as solubilized rCP-SCMV for purification using affinity chromatography. The recombinant His-tag fusion protein was purified by affinity column chromatography containing Ni-NTA resin equilibrated with the DNPI-10 buffer according to the manufacturer's instructions (Qiagen, Netherlands), with a ratio of 1:4. Unbound proteins from the washing process were stored for further analysis. Bounded-protein was eluted from the resin with buffer DNPI-250. Proteins from different steps were analyzed using SDS-PAGE in 10% acrylamide gel.

2.3. Purification by electroelution and quantification of purified protein

After separation by SDS-PAGE, the band representing high-molecular-weight complex was excised from the gel using a sharp, disposable blade and subjected to electroelution in the presence of SDS using an electroeluter (Bio-Rad, United States). The protein was electroeluted for 3 h at room temperature using an elution buffer (25 mM Tris–HCl, pH 8.3, 192 mM glycine, containing 0.1% (w/v) SDS), following the manufacturer's instructions. The recovered protein was dialyzed against a phosphate buffer saline (pH 8) overnight at 4°C to remove SDS from the protein mixture. The protein was recovered in a small amount and several samples were eluted simultaneously to produce a large amount of protein and concentrated using an Amicon Ultra-4 Centrifugal Filter Device (Merck, Germany). The purity of the protein was checked using SDS–PAGE and the presence of protein complexes were analyzed using 10% SDS-PAGE. The concentration of the protein was determined according to Bradford (1976). The optical density was noted at 595 nm and the amount of protein was calculated using BSA (bovine serum-albumin, Merck, Germany) as a standard protein.

3. Results

3.1. Detection of sugarcane mosaic virus and cloning of full-length CP-SCMV gene

PCR amplification was performed using the total RNA and full-length CP-SCMV gene from sugarcane variety Ps 881. This produced an amplicon fragment with a size of ± 1000 bp (Figure 1). This sugarcane variety was selected based on the presence of visual symptoms of mosaic disease, namely contrasting shades of greenish or yellowish chlorotic areas, especially in the young leaves. Sorghum plants from seedling were used to preserve the Indonesian SCMV isolate for further research by infecting sorghum leaves with leaf sap from Ps 881.

A CP-SCMV gene fragment was cloned into vector pJET1.2. The purpose of cloning to this plasmid was to store the targeted DNA fragment in a cloning vector hav-

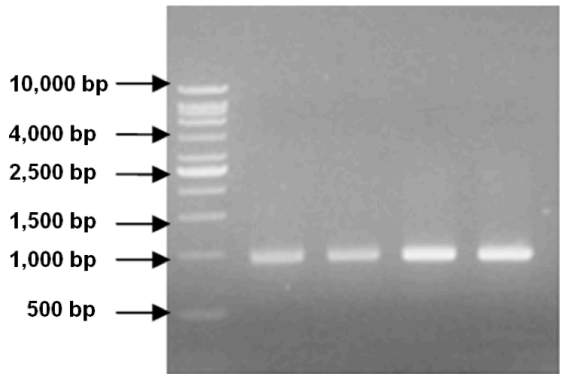

FIGURE 1 PCR amplification of full-length CP-SCMV gene from Ps 881 and infected sorghum with leaf sap from Ps 881. Lane 1 = 1 kb DNA marker; Lanes 2–3 = sorghum infected with sugarcane sap; Lanes 3–4 = Ps 881.

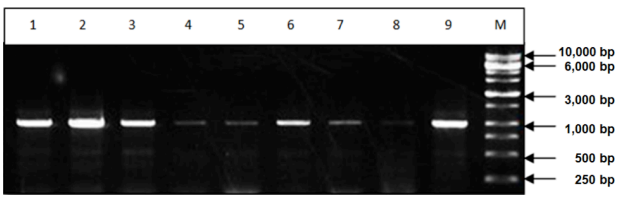

FIGURE 2 PCR amplification from *E. coli* DH5α transformant carrying full-length CP-SCMV gene, size = ± 1000 bp. M = DNA marker 100 kb; 1–9 = single colony of *E. coli* DH5α (colony number).

TABLE 1 Homology of nucleotide and amino acid sequence of coat protein of Indonesian SCMV isolate in comparison with other corresponding coat protein SCMV isolates stored in GenBank.

Isolate	Country of origin	% Homology		Accession number
		Nucleotide	Amino acid	
SCMV Indonesia*	Indonesia	-	-	-
SCMV ARG-130	Argentina	91	99.7	JX237868.1
SCMV ARG-345	Argentina	91	99.7	JX237865.1
SCMV Brisbane	Australia	90	99.3	AJ278405.1

*Isolate used in this study.

ing a T-end. After being transformed into *E. coli* DH5α, the CP-SCMV recombinant plasmid was isolated from a single colony of bacteria grown in selection media. A total of nine bacterial colonies of pJET1.2 CP-SCMV were verified using electrophoresis and visualized in 1% agarose gel with the control plasmid pJET1.2 (Figure 2). It was verified that all of the positive colonies were carrying the recombinant plasmid with a size of approximately 1000 bp DNA. Nucleotide and amino acid sequence comparisons with the coat protein of the Indonesian SCMV isolate showed the highest homology with SCMV from Argentina and Australia, respectively (Table 1). The differences in the amino acid between the Indonesian SCMV isolate and related SCMV homolog isolate from Argentina is 11 amino acids (data not shown).

Conserved region specific primers were designed by multiple alignment of the full-length CP-SCMV gene from several other SCMV isolates from GenBank (ARG-130, accession number: JX237868.1; ARG-345, accession number: JX237865.1; and Brisbane isolate, accession number: AJ278405.1). The nucleotide sequence was adjusted between the recognition site of the restriction enzymes, Xho1 and BamH1, in pET-28a (+) and conserved regions of the capsid protein gene so that it did not change nucleotide sequence encoding certain proteins and retained the nucleotide sequence in the expression vector. cDNA capsid protein SCMV in the pJET1.2 cloning vector (named pJET1.2-CP) was amplified using the aforementioned specific primers to obtain 925 bp size conserved region capsid protein fragments.

The CP-SCMV gene that was cloned in pJET1.2 was cut with two restriction enzymes (BamHI and XhoI) to obtain a cohesive end with expression vector pET-28a (+). The BamHI and XhoI restriction enzymes will only cut on the multicloning sites (MCS) and will fuse with histidine at both ends. MCS truncated parts will be replaced by a CP-SCMV conserved region. A total of eight colonies formed on selection media were confirmed using PCR with a T7 primer pair (Figure 3). Seven of these eight colonies positively contained the insertion gene of the conserved region of CP-SCMV. Colony C8 was reconfirmed again with PCR using the conserved region primers (Figure 4). The results showed that colony C8 had a single band DNA 925 bp in size.

3.2. *Expression and purification of rCP-SCMV*

The protein band exhibited a similar appearance in terms of thickness at all levels of induction; however, lower concentrations of IPTG were found to be suitable for high levels of expression (Figure 5). The optimum concentration of IPTG at 0.1 mM for 5 h was used for over-expression. According to Akbari et al. (2015), which might be degrade recombinant protein, and potentially toxic to bacterial cell. The results of SDS-PAGE showed that the recombinant protein was expressed with a molecular weight of about 40 kDa. The inserted conserved region of the CP-SCMV

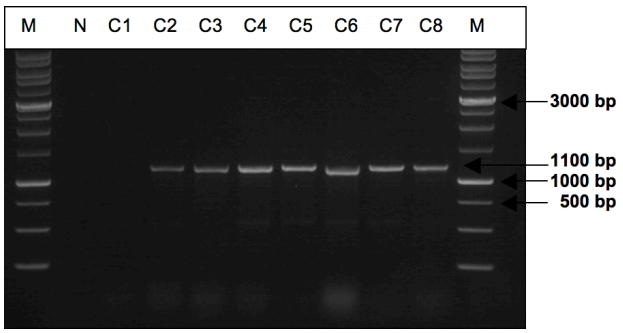

FIGURE 3 PCR amplification from *E. coli* BL21 transformant carrying conserved region of CP-SCMV gene using T7 promoter and T7 terminator primers, size = ± 1100 bp. M = DNA marker 1 kb; C1–C8 = colony number of *E. coli*; N = negative control.

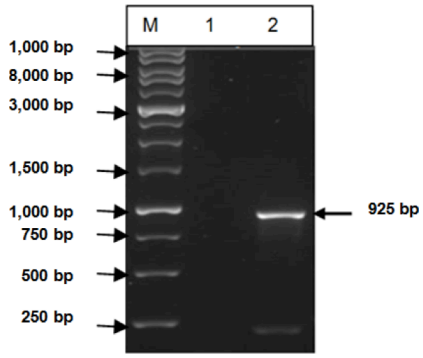

FIGURE 4 PCR amplification of colony C8 using conserved region primer, 925 bp. M = DNA marker 1 kb; 1 = negative control; 2 = colony C8.

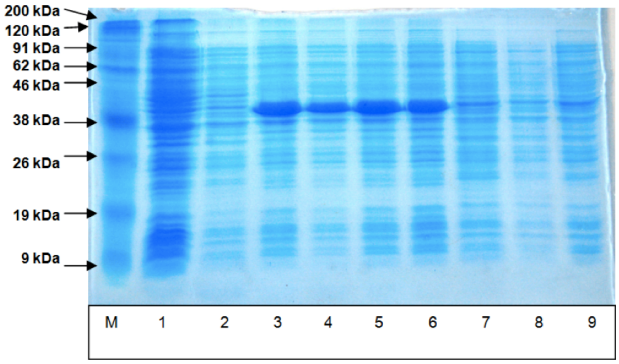

FIGURE 5 Results of recombinant CP-SCMV expression. M = marker protein; 1 = BL 21 wild type (negative control *E. coli*); 2 = pET-28a without inserted gene (negative control plasmid); 3 = pellet BL 21 pET-28a CP-SCMV; IPTG 0.1 mM, 5 hours lysozyme, 20 times dilution; 4 = pellet BL 21 pET-28a CP-SCMV, IPTG 0.5 mM, 5 hours lysozyme, 20 times dilution; 5 = pellet BL 21 pET-28a CP-SCMV, IPTG 1.0 mM, 5 hours lysozyme, 20 times dilution; 6 = pellet BL 21 pET-28a CP-SCMV, IPTG 0.1 mM, 3 hours lysozyme, 20 times dilution; 7 = supernatant BL 21 pET-28a CP-SCMV, IPTG 0.1 mM, 5 hours lysozyme, no dilution; 8 = supernatant BL 21 pET-28a CP-SCMV, IPTG 0.5 mM, 5 hours lysozyme, no dilution; 9 = supernatant BL 21 pET-28a CP-SCMV, IPTG 1.0 mM, 5 hours lysozyme, no dilution.

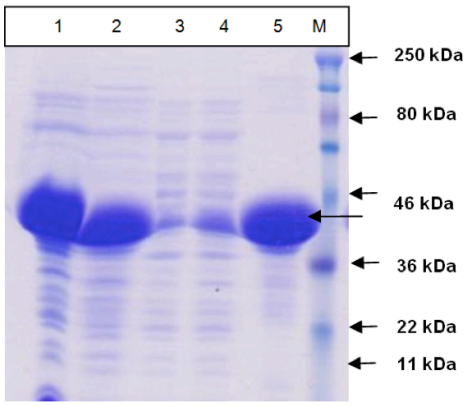

FIGURE 6 Electrophoresis of recombinant protein purification by Ni-NTA resin. 1 = supernatant (before purification); 2 = first filtration; 3 = second filtration; 4 = protein from washing step; 5 = purified protein; M = protein marker.

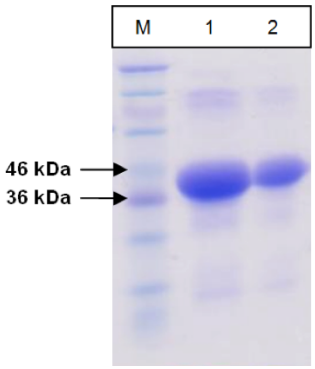

FIGURE 7 Results of protein concentration. 1 = after concentration; 2 = before concentration.

gene was located below the target DNA (downstream) and in frame with the sequence encoding six histidine residues that function as a metal binding domain in protein translation. Their N-terminal fusion peptide in the vector will add 3–4 kDa protein expressed. Thus, the protein molecular weight expression resulting from the recombinant construct of CP-SCMV was approximately 40 kDa (Figure 6). The recombinant coat proteins of other *Potyviruses* (papaya ring spot virus, grapevine leafroll associated virus 3) were also reported to have the same molecular weights (Fajardo et al. 2007; Sreenivasulu and Gopal 2010).

To meet the quality requirements of a proper antigen, the isolated protein migrated in a polyacrylamide gel was then refined using electroelution. Protein specificity from electroelution resulted in a highly purified and specific protein with a molecular weight of 40 kDa (Figure 7). The quality of protein concentration was analyzed by 10% SDS-PAGE to compare the results before and after concentration using a column filter (Figure 7). This step was carried out to increase protein concentration in order to minimize the volume when being injected into the animal sample for antibody production.

Based on protein quantification, the final concentration obtained after column filter treatment was 16,184 mg/mL, or about 80 times compared with the original (without concentrated treatment). According to the Institutional Animal Care and Use Committee (2016), the optimum concentration of antigen for antibody induction is 50–1000 µg for rabbits and 10–200 µg for mice.

4. Discussion

Mosaic disease is known as a common viral disease in sugarcane fields amongst sugarcane producing countries. A previous survey by (Addy et al. 2017) in commercial sugarcane fields in East Java, Indonesia, proved that some sugarcane cultivars are showing mosaic symptoms on leaves with different incidence and severity levels, and that the causal agent for these symptoms is SCMV. A monitoring program using an immunoassay as a routine procedure in nursery and commercial fields is necessary to manage and control the spread of viral infections. Immunoassays are commonly assembled using antibodies prepared against recombinant viral proteins.

PCR cloning is a fast method for cloning genes and is often used to obtain a higher yield than the DNA library method. This technique is also commonly used to obtain DNA fragments that are not available in large quantities. Besides these benefits, this research used the PCR cloning technique because the plant virus had been known as easily mutated particles. Cloning genes from other plants capsid proteins of *Potyviridae* has been widely reported, including cowpea (Koohapitagtam and Nualsri 2013), potato (Abdel-Salam et al. 2013), sweet pepper (Badr et al. 2012), grapes (Fajardo et al. 2007), and plum (Crescenzi et al. 1997). Hema et al. (2003) has been successfully increasing the coat protein gene expression of sugarcane streak mosaic virus (SCSMV) in *E. coli* strain BL21 (DE3) using pRSET-A as an expression vector. Studies on SCMV by Mohammadi and Hajieghrari (2009) and Haider et al.

(2011) also proved that the amplicon size of CP-SCMV was ± 900 bp.

To store the targeted DNA fragments in a cloning vector, we used the pJET1.2/blunt cloning vector (Thermo Fisher Scientific, patent publication: US 2009/0042249 A1, Genbank accession number EF694056.1) and cloned the PCR product into the linearized vector. This vector contains a lethal gene (*eco47IR*) that is activated in case the vector becomes circularized. However, if the PCR product is cloned into the cloning site within the lethal gene, the latter is disrupted and allows bacteria to form a colony upon transformation. The inserted PCR product disrupts the toxic gene *eco47IR*, there is no need for blue/white screening, works for both blunt end and single-base overhang-containing PCR products, up to 10 kb PCR product size (Hoseini and Sauer 2015). The cloning system in vector pJET1.2-CP was cut with two different kinds of restriction enzymes, BamHI and XhoI, which aimed to place the exact direction of the attached inserted gene CP-SCMV in the expression vector, pET-28a (+).

The pET series are widely used in laboratory scale for inducible protein production (Studier et al. 1990). The system is a highly effective set of vectors yet developed for the subcloning and over-expression of recombinant proteins in *E. coli*. Genes that encode a protein of interest are generally inserted into a restriction enzyme site located at multiple cloning regions downstream of a T7 promoter for IPTG-inducible transcription by the T7 RNA polymerase (Gay et al. 2014). The expression vector pET-28a (+) has an additional fusion tag peptide (6x Histidin) expressed in *E. coli* that increases expression, solubility, stability, and purity (Carson et al. 2007).

The structural integrity of the expressed protein is one of the main objectives in recombinant protein production. This is owed partly to the original protein structure having a key role in inducing specific antibodies. The purification process must also maintain the antigenic epitope and the structure of the recombinant protein. Electroelution is used to remove a specific protein of interest from an electrophoresis gel by applying an electric current (Thanasarasakulpong et al. 2016). Purification of the recombinant protein was accomplished using electroelution, which enabled a rapid and quantitative elution of proteins from denaturing gels. This system also has the benefit of having a high loading capacity of sample proteins while allowing easy monitoring of the elution process.

An earlier study using electroelution for purification of the target protein showed that this method provides effective purification to protect immunogenicity of the target protein (Barrell et al. 2004; Kaur et al. 2013). On the other hand, affinity chromatography is regarded as an effective purification method in separating low molecular weight proteins from crude protein samples (Barrell et al. 2004; Kaur et al. 2013). However, Luo et al. (1997) and Rimler (2001) mentioned that this chromatography process results in a change of the outer membrane structure of the recombinant protein and affects its immunogenicity. To overcome this issue, many researchers have combined affinity chromatography with a purification method based on electroelution to avoid contamination by other proteins (Saraswat et al. 2013; Vázquez-Iglesias et al. 2017). In this study, electroelution was used to purify the rCP-SCMV from cell lysate to protect the immunogenicity of the protein and prevent the existence of contaminants.

Purification by electroelution is an adequate and replicable method that could be used as a regular method to elute multiple protein bands, enabling reasonable savings in time. It is simple, fast, and cost-effective; it does not need an expensive apparatus and reagents, nor does it require prior knowledge of the protein characteristics. This technique is not restricted to soluble proteins but to membrane-bound proteins, as well, and it is applicable to raising antibodies (Ohhashi et al. 1991). In addition, purification of proteins, particularly toxins, based on electroelution has been successfully achieved in earlier studies (Borowiec et al. 2016).

In this study, we successfully cloned the CP-SCMV gene from an Indonesian isolate and efficiently expressed in a bacterial expression system, and this will be used as an antigen for the production of polyclonal antibodies for further research. The cloning strategy used for construction of the CP-SCMV protein resulted in the addition of 3-4 kDa of the N-terminus. Thus, the molecular weight of the protein expression resulting from recombinant CP-SCMV was approximately 40 kDa. Hence, the coat protein was expressed as a fusion protein with an estimated molecular weight of about 40 kDa, which is about 4 kDa more than the expected molecular weight of 36 kDa. Based on these results, recombinant CP-SCMV was successfully produced and expressed in the recombinant bacterial expression system. Furthermore, rCP-SCMV in highly purified, specific, and high concentration form was obtained with the use of a purification technique based on eletroelution followed by concentrated protein. Comparing our results with those of other similar studies (Abdel-Salam et al. 2014; Thanasarasakulpong et al. 2016), we could readily gather a milligram amount of proteins using this method (16,184 mg/mL), which is sufficient to raise antibodies. Thus, the rCP-SCMV obtained from this study is suitable as a proper antigen.

Acknowledgments

This study was financially supported by a Post Graduate Research Grant from Airlangga University. Authors are grateful to Airlangga University and PT Perkebunan Nusantara XI, as well as the University of Jember (Prof. Bambang Sugiharto) for providing laboratory facilities.

Authors' contributions

ND and WD designed this study. ND, WDS, and SPA wrote the manuscript. NTA and SW carried out the laboratory work. WDS and WD analyzed the data. ND research funding. NTA and WDS, authors contributed to the final version of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

References

Abdel-Salam AM, El-Attar AK, Gambley CF. 2014. Production of polyclonal antisera to a recombinant coat protein of *Potato virus Y* expressed in *Escherichia coli* and its application for immuno diagnosis. Int J Virol. 10(1):1–16. doi:10.3923/ijv.2014.1.16.

Abdel-Salam AM, El-Attar AK, Soliman A. 2013. The use of native and denatured recombinant coat protein forms for induction of good quality antisera for *Potato virus X* and *Potato leaf roll* virus. Am J Res Commun. 1(17):70–86.

Addy H, Nurmalasari, Wahyudi A, Sholeh A, Anugrah C, Iriyanto F, Darmanto W, Sugiharto B. 2017. Detection and response of sugarcane against the infection of *Sugarcane mosaic virus* (SCMV) in Indonesia. Agronomy. 7(3):50. doi:10.3390/agronomy7030050.

Akbari V, Sadeghi HMM, Jafarian-Dehkordi A, Chou CP, Abedi D. 2015. Optimization of a single-chain antibody fragment overexpression in Escherichia coli using response surface methodology. Res Pharm Sci. 10(1):75–83.

Anwar S. 2005. Impact of sugarcane disease on cane and sugar yield. Proceedings of the 3rd Agricultural Biotechnology Workshop; Faisalabad, Pakistan. Faisalabad: Agricultural Biotechnology Research Institute.

Badr MAS, Zein AB, Salwa N, Khalifa MAA. 2012. Antiserum production and control measures of *Pepper mottle potyvirus* using different applications. Int J Virol. 8(1):71–80. doi:10.3923/ijv.2012.71.80.

Barrell PJ, Liew OW, Conner AJ. 2004. Expressing an antibacterial protein in bacteria for raising antibodies. Protein Expression Purif. 33(1):153–159. doi:10.1016/j.pep.2003.08.026.

Borowiec M, Gorzkiewicz M, Grzesik J, Walczak-Drzewiecka A, Salkowska A, Rodakowska E, Steczkiewicz K, Rychlewski L, Dastych J, Ginalski K. 2016. Towards engineering novel PE-based immunotoxins by targeting them to the nucleus. Toxins. 8(11):321. doi:10.3390/toxins8110321.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72(1–2):248–254.

Carson M, Johnson DH, McDonald H, Brouillette C, DeLucas LJ. 2007. His-tag impact on structure. Acta Crystallogr, Sect D: Biol Crystallogr. 63(3):295–301. doi:10.1107/S0907444906052024.

Crescenzi A, d'Aquino L, Nuzzaci M, Ostuni A, Bavoso A, Comes S, Stradis AD, Piazzolla P. 1997. Production of strain specific antibodies against a synthetic polypeptide corresponding to the N-terminal region of the *Plum pox potyvirus* coat protein. J Virol Methods. 69(1-2):181–189. doi:10.1016/s0166-0934(97)00158-4.

Damayanti TA, Putra LK. 2011. First occurrence of *Sugarcane streak mosaic virus* infecting sugarcane in Indonesia. J Gen Plant Pathol. 77(1):72–74. doi:10.1007/s10327-010-0285-7.

Fajardo TVM, Barros DR, Nickel O, Kuhn GB, Zerbini FM. 2007. Expression of *Grapevine leafroll*-associated virus 3 coat protein gene in *Escherichia coli* and production of polyclonal antibodies. Fitopatol Bras. 32(6):496–500. doi:10.1590/S0100-41582007000600007.

Gay G, Wagner DT, Keatinge-Clay AT, Gay DC. 2014. Rapid modification of the pET-28 expression vector for ligation independent cloning using homologous recombination in *Saccharomyces cerevisiae*. Plasmid. 76:66–71. doi:10.1016/j.plasmid.2014.09.005.

Gulati-Sakhuja A, Sears JL, Nunez A, Liu HY. 2009. Production of polyclonal antibodies against *Pelargonium zonate spot virus* coat protein expressed in *Escherichia coli* and application for immunodiagnosis. J Virol Methods. 160(1-2):29–37. doi:10.1016/j.jviromet.2009.04.005.

Haider MS, Afghan S, Riaz H, Tahir M, Javed MA, Rashid N, Iqbal J. 2011. Identification of two *Sugarcane mosaic virus* (SCMV) variants from naturally infected sugarcane crop in Pakistan. Pak J Bot. 43(2):1157–1162.

Hema M, Kirthi N, Sreenivasulu P, Savithri HS. 2003. Development of recombinant coat protein antibody based IC-RT-PCR for detection and discrimination of *Sugarcane streak mosaic virus* isolates from Southern India. Arch Virol. 148(6):1185–1193. doi:10.1007/s00705-003-0015-y.

Hoseini SS, Sauer MG. 2015. Molecular cloning using polymerase chain reaction, an educational guide for cellular engineering. J Biol Eng. 9(1):2. doi:10.1186/1754-1611-9-2.

Institutional Animal Care and Use Committee. 2016. Administering complete freund's adjuvant (CFA) and other adjuvants. Atlanta: Emory University.

Kaur U, Khurana S, Saikia UN, Dubey ML. 2013. Immunogenicity and protective efficacy of heparan sulphate binding proteins of *Entamoeba histolytica* in a guinea pig model of intestinal amoebiasis. Exp Parasitol. 135(3):486–496. doi:10.1016/j.exppara.2013.08.011.

Khalil SM, Salama MI, Abdel-Hamid A I, El-Din HAN, Sadik AS. 2007. Effective detection of *Banana bunchy top nanovirus* using DNA probes. Pak J Biotechnol. 4(1-2):113–118.

Khan S, Jan AT, Mandal B, Haq QMR. 2012. Immunodiagnostics of *Cucumber mosaic virus* using antisera developed against recombinant coat protein. Arch Phytopathol Plant Prot. 45(5):561–569. doi:10.1080/03235408.2011.588043.

Khatabi B, He B, Hajimorad MR. 2012. Diagnostic potential of polyclonal antibodies against bacterially ex-

pressed recombinant coat protein of *Alfalfa mosaic virus*. Plant Dis. 96(9):1352–1357. doi:10.1094/pdis-08-11-0683-re.

Koohapitagtam M, Nualsri C. 2013. Production of polyclonal antibodies specific to the recombinant coat protein of *Blackeye cowpea mosaic* virus and its use in disease detection. Kasetsart J Nat Sci. 47(4):603–613.

Luo Y, Glisson JR, Jackwood MW, Hancock RE, Bains M, Cheng I, Wang C. 1997. Cloning and characterization of the major outer membrane protein gene (ompH) of *Pasteurella multocida* X-73. J Bacteriol. 179(24):7856–7864. doi:10.1128/jb.179.24.7856-7864.1997.

Mohammadi MR, Hajieghrari B. 2009. *Sugarcane mosaic virus*: The causal agent of mosaic disease on sorghum (*Sorghum bicolor* l.) in Tehran province of Iran. Afr J Biotechnol. 8(20):5271–5274.

Ohhashi T, Moritani C, Andoh H, Satoh S, Ohmori S, Lottspeich F, Ikeda M. 1991. Preparative high-yield electroelution of proteins after separation by sodium dodecyl sulphate—polyacrylamide gel electrophoresis and its application to analysis of amino acid sequences and to raise antibodies. J Chromatograph A. 585(1):153–159. doi:10.1016/0021-9673(91)85069-r.

Rimler RB. 2001. Purification of a cross-protective antigen from *Pasteurella multocida* grown in vitro and in vivo. Avian Dis. 45(3):572–580. doi:10.2307/1592897.

Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Saraswat M, Musante L, Ravidá A, Shortt B, Byrne B, Holthofer H. 2013. Preparative purification of recombinant proteins: current status and future trends. BioMed Res Int. 2013:1–18. doi:10.1155/2013/312709.

Shukla DD, Frenkel MJ, McKern NM, Ward CW, Jilka J, Tosic M, Ford RE. 1992. Present status of the sugarcane mosaic subgroup of potyviruses. In: Barnett OW, editor. Potyvirus taxonomy. volume 5 of *Archives of Virology (Supplementum 5)*. Vienna: Springer Vienna. p. 363–373. doi:10.1007/978-3-7091-6920-9_39.

Sreenivasulu M, Gopal DVR. 2010. Development of recombinant coat protein antibody based IC-RT-PCR and comparison of its sensitivity with other immunoassays for the detection of *Papaya ringspot virus* isolates from India. Plant Pathol J. 26(1):25–31. doi:10.5423/ppj.2010.26.1.025.

Studier FW, Rosenberg AH, Dunn JJ, Dubendorff JW. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. 185:60–89. doi:10.1016/0076-6879(90)85008-c.

Thanasarasakulpong A, Poolperm P, Tangjitjaroen W, Varinrak T, Sawada T, Pfeiffer D, Sthitmatee N. 2016. Comparison of the effect of two purification methods on the immunogenicity of recombinant outer membrane protein h of *Pasteurella multocida* serovar a: 1. Int J Virol. 2016:1–7. doi:10.1155/2016/2579345.

Vázquez-Iglesias L, Estefanell-Ucha B, Barcia-Castro L, de la Cadena M, Álvarez-Chaver P, Ayude-Vázquez D, Rodríguez-Berrocal FJ. 2017. A simple electroelution method for rapid protein purification: isolation and antibody production of alpha toxin from *Clostridium septicum*. PeerJ. 5:e3407. doi:10.7717/peerj.3407.

Xu DL, Park JW, Mirkov TE, Zhou GH. 2008. Viruses causing mosaic disease in sugarcane and their genetic diversity in southern China. Arch Virol. 153(6):1031–1039. doi:10.1007/s00705-008-0072-3.