ORIGINAL ARTICLE

Purity of maltose-binding protein – Recombinant streptavidin expressed in *Escherichia coli* BL21 (pD861-MBP: 327892)

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ABSTRACT

Nearly 95% of streptavidin which is expressed in *Escherichia coli* found as an inclusion body. Protein expressed in an inclusion body form requires further steps for the folding process related to its purification. Whereas the purity level of the recombinant streptavidin is very crucial mainly for the specification test in diagnostic system. In this study, we designed synthetic gene of streptavidin to be fused with maltose-binding protein (MBP) gene to enhance its solubility when expressed in *E. coli* BL21 (pD861-MBP: 327892) and purified using amylose resin with gradient column buffer. Based on the SDS-PAGE characterization, the majority of recombinant streptavidin was found in soluble than that of insoluble form. Recombinant streptavidin was found at its suitable size at 56.6 kDa in the soluble protein fraction with a concentration of 537.42 mg/L. The purest fraction of streptavidin recombinant was obtained at the 58th fraction in a concentration of 0.86 mg/L with purity level of 98.77%. Compared to the initial crude protein extract, the level of purity is lower, 6.03%. In summary, the MBP purification method improves the purity level and enhances the solubility of the recombinant streptavidin.

Key words: *Escherichia coli* BL21 (pD861-MBP: 327892), maltose-binding protein, recombinant, soluble, streptavidin

INTRODUCTION

Immunochromatography generally uses immunoglobulin G antibodies as analyte detection because it can bind specifically to antigens and can be strongly adsorbed on nitrocellulose membranes. This attachment is carried out by direct physical adsorption which allows the orientation of antibodies at random so that the active site of antibody binding can be blocked.[1,2] Therefore, it is necessary to develop an antigen-binding agent that can immobilize target proteins on nitrocellulose membranes with proper orientation and high homogeneity. The streptavidin-biotin system can be chosen as a strong candidate as a protein immobilization solution on nitrocellulose membranes because it has several advantages such as, until now the bond between streptavidin-biotin is the strongest noncovalent bond (Kd ~10–14), resistant to organic solvents, detergents, proteolytic enzymes, and extreme changes in temperature and pH.[3,4] Streptavidin can be conjugated with other proteins or tagged with various

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detection reagents without losing its ability to bind biotin.[4] However, commercially available recombinant streptavidin costs are expensive and must be imported. Therefore, the need of streptavidin as an important supported diagnostic component must be created independently.

In this study, the recombinant streptavidin gene was expressed on *Escherichia coli* BL21 (DE3). However, nearly 95% of streptavidin which is expressed in *E. coli* found in an insoluble form or is commonly known as an inclusion body.[5] This potential problem can affect the structural conformation of streptavidin in recognition of its binding to biotin. The stability of the structure of streptavidin greatly determines the success of its binding to biotin. In addition, protein expressed in an inclusion body form requires further steps for the folding process related to its purification. The solution to overcome this problem is by fusing recombinant proteins with compounds that have specific solubility to increase their stability to avoid aggregation.[5-8]

Maltose-binding protein (MBP) is used as a fusion protein in recombinant streptavidin expression because of its several advantages over other fusion proteins which can support the purification process with only one stage using amylose resin and gives higher yields.[6] MBP can be chosen as a fusion protein that improves the protein solubility and is used to prevent the formation of inclusion bodies, especially in recombinant proteins, that have low solubility when expressed on *E. coli*. Recombinant proteins that have not completely folded will undergo several binding processes and release from MBP. MBP can facilitate interactions between recombinant proteins that have not yet been completely folded with one or more endogenous chaperons.[9,10]

MATERIALS AND METHODS

Materials

The materials used are Luria-Bertani agar (Merck), SDS-PAGE components, ethanol, synthetic gene pD861-MBP: 327892 (synthesized in ATUM, California, USA), L-rhamnose (Sigma-Aldrich), amylose resin (BioLabs), and other common chemicals for SDS-PAGE.

Construction of synthetic recombinant streptavidin genes

In this study, the synthetic gene encoded recombinant streptavidin was obtained from www. ncbi. nlm. nih. gov with access number KT344129.1. The amino acid sequence of this protein was composed of:

Menlyfqsaeagitgtwynqlgstfivtag Adgaltgtyesavgnaesr Yvltgryds Apatdgsgtalgwtvawknnyrna Hsattwsgqyvgga Earintqwllt Sgtteanawkstlvghdtftkvkpsaasdykddddk.

In this amino acid strand, there is an ENLYFQS sequence which is the specific side to be cut by TEV protease enzyme. Therefore, the recombinant streptavidin can be purified from MBP protein as the fusion tag protein. In addition, there is a DYKDDDK sequence which is FLAG marker amino acid sequence that serves to isolate recombinant streptavidin or can be used for quantification using enzyme-linked immunosorbent assay method. Overall, the gene construction expressed containing 435 bp can be illustrated as shown in the Figure 1.

Preparation of *Escherichia coli* competent cells and transformation

One Ose of E. coli from slant agar was inoculated into a 5 mL of nonNaCl Luria Bertani (LB) liquid medium and incubated (20 h, 37°C, 180 rpm). A volume of 1 mL of bacterial suspension was reinoculated in 50 mL of liquid LB. The media was incubated for approximately 4 h, 37°C, and 180 rpm until its OD600 achieved 0.8–1. After that, the bacterial suspension was kept on ice for 10–15 min and centrifugated (5 min, 5000 rpm, and 4°C) to isolate the cell pellets. The transformation of 5 µL pD861-MBP: 327892 was conducted using the electroporation method.[11]

Recombinant streptavidin expression

One Ose of transformant colony was inoculated into 5-mL liquid media which carrying 30-µL kanamycin (25 mg/mL) then incubated (37°C, 180 rpm for 16–18 h). Furthermore, 1 mL of transformant cell culture was inoculated into 99 mL of LB liquid media containing kanamycin and 1% glucose. Then, the culture was incubated at °C, 200 rpm, and 180 rpm until OD600 reached 0.6–1 (approximately need 1–2 h). A volume of 1-mL cell culture was taken as t_0 (before the induction of L-rhamnose) and put in a microtube. Cell pellets and supernatants were separated by centrifugation at a rate of 6.000 g at 4°C for 20 min. The culture was added with L-rhamnose (4 mM) and re-incubated at 37°C, 180 rpm for 5 h. A volume of 1-mL transformant culture was taken into a microtube, then centrifuged at a rate of 6.000 g, 4°C, and 20 min to separate the supernatant. The supernatant was decanted and transferred it into a new microtube. Meanwhile, the cell pellets in a weight of 1 g were suspended in 4 mL of ethylenediaminetetraacetic acid Tris-Cl buffer to be lysed using a sonicator for 15 min (2 s on/2 s off). The lysis cell suspension was centrifuged at 10.000 g at 4°C for 30 min; then, the supernatant was taken and transferred to a new microtube as a soluble fraction (SF). Cell pellets were added with 70 µL 8 M urea, then heated at 95°C for 15 min, and centrifuged at 10,000 g at 4°C for 10 min. The supernatant was transferred to a new microtube as insoluble fraction (IF). The protein fractions then were characterized by SDS-PAGE method.[12]

Figure 1: Recombinant streptavidin gene mapping. Notes: T = specific side to be cut by TEV protease; F = FLAG marker

Purification of streptavidin recombinant proteins

A volume of 3-mL amylose resin was added into the One Med Disposable Syringe 5 cc column then the resin was washed with 15-mL column buffer (5 CV). The resin was equilibrated with 24-mL column buffer (8 CV) and then 4,09 mL of sample was added and eluted with 36-mL column buffer (12 CV), then re-eluted with 36-mL column buffer containing 10-mM maltose (12 CV). The fraction was collected and characterized by UV–Vis spectrophotometer at a wavelength of 280 nm. The peaks of fraction were characterized by SDS-PAGE, and their protein total content was quantified using Lowry method and ImageJ analysis program. Characterization of recombinant streptavidin in SF was carried out using the SDS-PAGE method.

RESULTS AND DISCUSSION

We produced streptavidin by expressing the synthetic gene encoding streptavidin in *E. coli* BL21 (DE3). The synthetic gene was expressed better than that of identical native gene. In addition, the advantages when using synthetic genes are rapid process, no cell extraction needed and dependable access to obtain cDNA target sequences in gene bank. Moreover, the level of synthetic gene expression can be regulated by designing the expression vector component containing the target gene. Promoter, enhancer elements, signals, and restriction sites of expression system can be incorporated into the vector to elevate the yield of protein recombinant correctly.[13] Unfortunately, the expression sometimes leads to the formation of insoluble or misfolded proteins. However, this problem could be rectified by choosing the suitable codon usage to be expressed in the cell host.[14,15] From the graph in Figures 2 and 3 shown that the adaptive percentage of the streptavidin original codon <100% in *E. coli*. These differences will not be optimal to be read and expressed in *E. coli*.[16]

In Figure 4, the optimized codon sequence of streptavidin gene has changed to 100%, consistent with the codon preference in *E. coli*. Then, the optimized streptavidin gene sequence was inserted in the expression vector, shown in Figure 5. This plasmid vector consists of kanamycin-resistant antibiotic gene marker, rhaBAD promoter, strong RBS, initiator of Ori_pUC replication, and MBP as fusion protein that can increase the solubility of recombinant streptavidin. This plasmid vector can then be directly transformed into the host *E. coli* BL21 (DE3).

As illustrated in Figure 6, in the Petri dish A (positive control), the pure strain of *E. coli* BL21 (DE3) colonies was found on the growth media without kanamycin. In contrast to Petri dish B, when the pure strain was growth in the media containing kanamycin, then there was no colony found. However, the

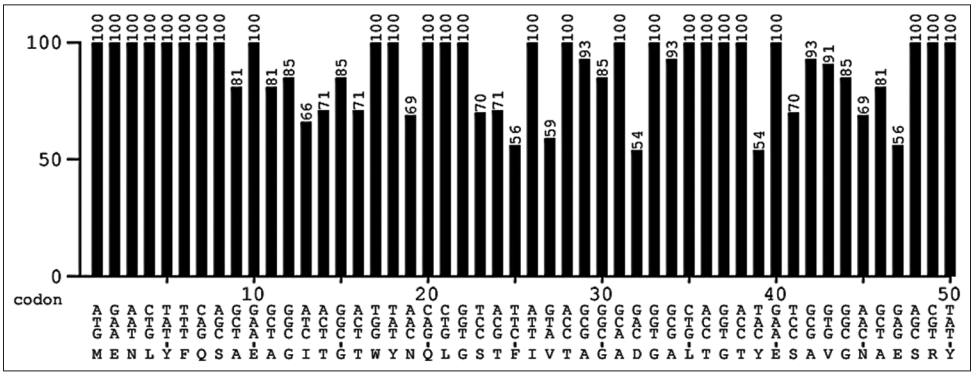

Figure 2: Adaptive percentage of recombinant streptavidin codons to codon preferences of *Escherichia coli*

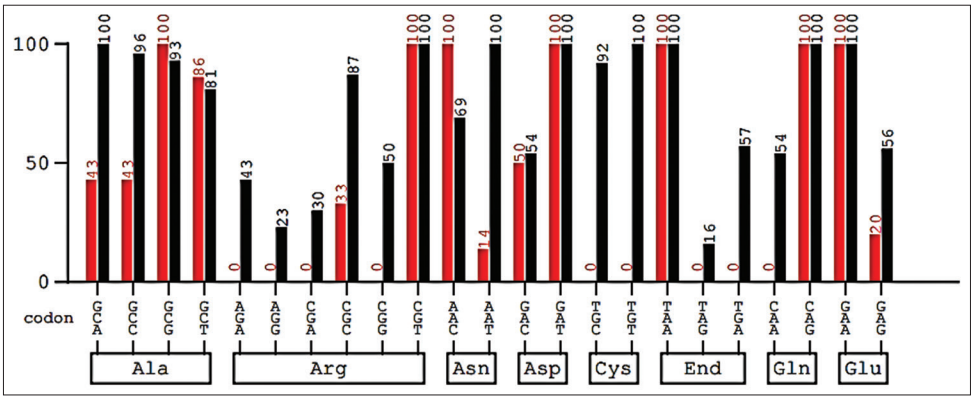

Figure 3: Amino acids that should change nitrogen bases sequences. The red bar diagram shows the adaptive percentage of recombinant streptavidin codons and the black bar diagram shows the adaptive percentage of *Escherichia coli* codons

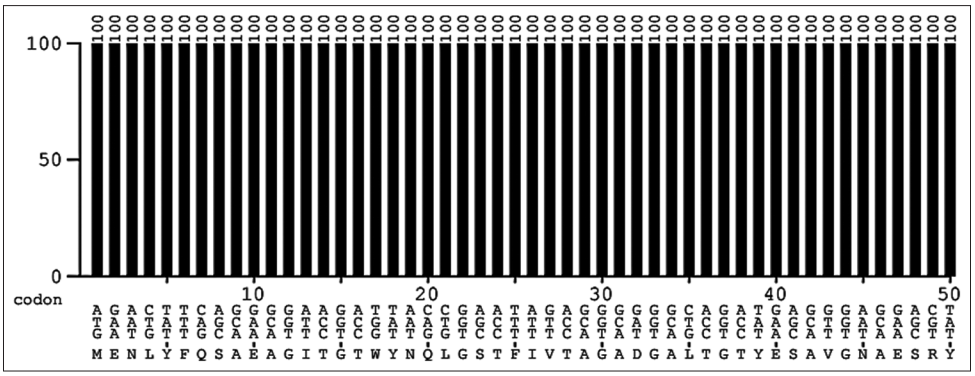

Figure 4: Codon adaptive percentage graphic after codon optimization using GCUA

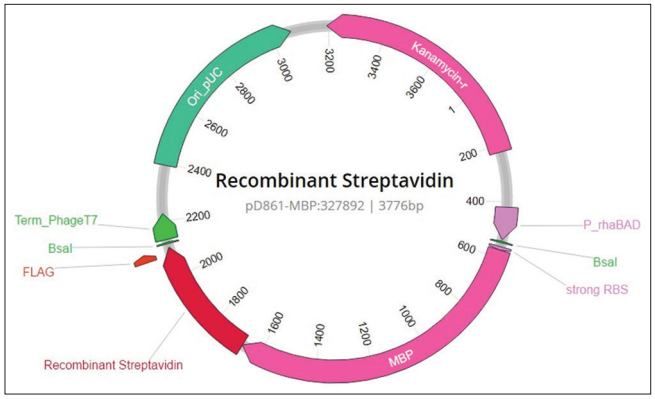

Figure 5: Map of pD861-MBP plasmids

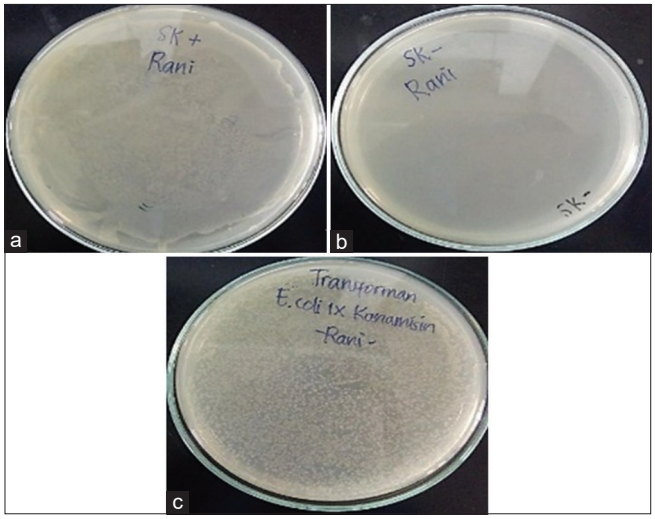

Figure 6: The results of the transformation (a) negative control (b) positive control (c) transformant

transformants colonies are able to grow well in the media contains kanamycin antibiotics, appeared in Petri dish C.

Figure 7 demonstrated that the growth curve showed the lag phase of *E. coli* BL21 (DE3) (pD861-MBP) occurring at 0–1th h. Then, at the 2nd–17th h is the log phase or exponential phase where *E. coli* begins to divide exponentially and enter the logarithmic phase. The induction process is carried out in the exponential phase, based on the procedure suggested by ATUM, the induction is carried out when the OD600 value is in the range of 0.6–0.8. The growth curve showed that OD600 values began to enter the range between the 2nd and 3rd h. Production curve of recombinant streptavidin expression, shown in Figure 8. As the theory, most of recombinant streptavidin was expressed in the SF, shown in Figure 9. The highest protein content in SF at 5th h in a concentration of 0.532238 mg/mL.

The expression fraction in the form of IF, SF, and M was analyzed to determine the presence of recombinant streptavidin. Recombinant streptavidin with its suitable size (56 kDa) was detected, presented in Figure 10, and the estimated recombinant streptavidin content of the expression was 537.42 mg/L. Theoretically, the recombinant protein is mostly found as the SF in *E. coli* due to the influence of MBP fusion protein that can increase the solubility of recombinant protein and minimize the formation of inclusion in IF.[17-20]

The streptavidin recombinant protein was purified by the affinity chromatography method, shown in Figure 11. The 3rd–15th fraction was detected in 280 nm and it was recharacterized using SDS-PAGE to confirm the purity level of the purification method, as shown in Figure 12. The analysis showed that the level of streptavidin recombinant purity was not good enough. Table 1 and Figure 13 demonstrated the purest fraction (98.77%) was at 58th fraction and the recovery was 6.80%.

Amylose resin is used as an affinity matrix to separate target proteins fused with MBP by the principle of gravity-flow column. Thus, the target protein can be isolated specifically by one purification step. In this study, the purest fraction was fraction 58 in purity level at 98.77% and its recovery is 6.80%. The purity level of the isolated protein using amylose resin can be affected by several factors, such as the weak binding due to the production of amylase which can interrupt amylose thus we added the glucose into the growth medium to restrain amylase expression.

Table 1: Purification results

Sample	Vol (mL)	RSA content (mg/mL)	Protein total content (mg/m)	Total RSA content (mg)	Total protein content (mg)	Specific content	Purity (%)	Purity (fold)	Recovery (%)
CE	4.09	3.06±0.001	50.68±0.002	12.49±0.003	207.13±0.002	0.06±0.000	6.03±0.000	1.00	100.00±0.000
Fraction 58	1	0.85±0.000	0.86±0.001	0.85±0.000	0.86±0.001	0.99±0.001	98.77±0.000	16.37	6.80±0.000

CE: Crude extract, RSA: Recombinant Streptavidin

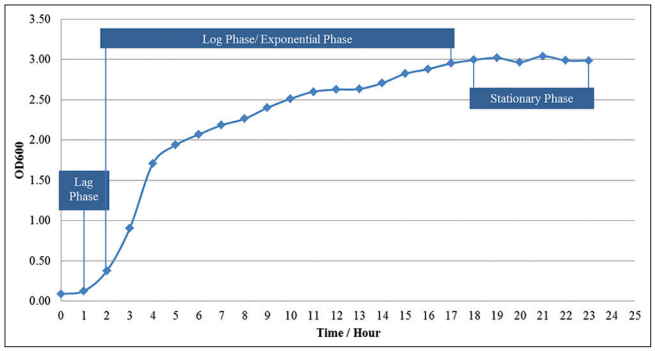

Figure 7: *Escherichia coli* BL21(DE3) (pD861-MBP) growth curve

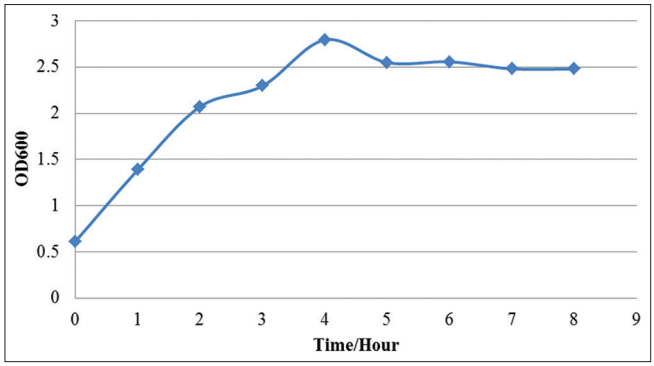

Figure 8: *Escherichia coli* BL21(DE3) (pD861-MBP) production curve

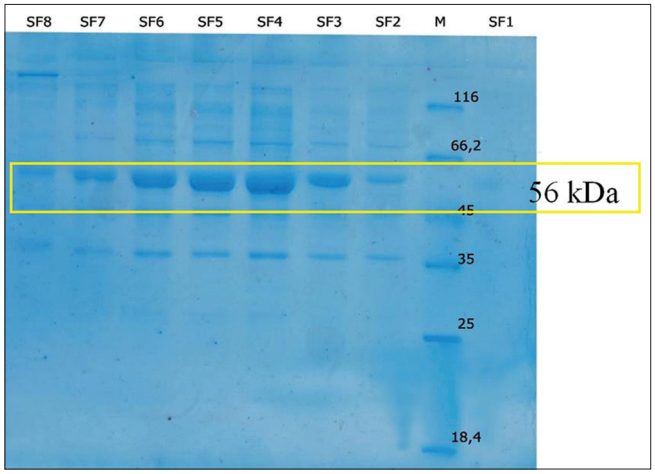

Figure 9: SDS-PAGE electropherogram of soluble fraction streptavidin recombinant (56 kDa)

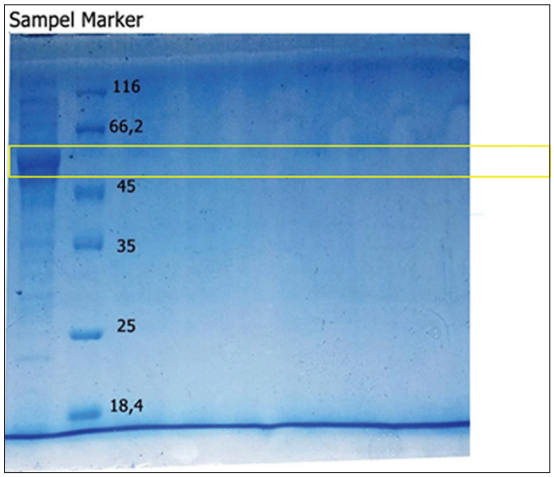

Figure 10: SDS-PAGE electrophoregram of recombinant streptavidin soluble fraction

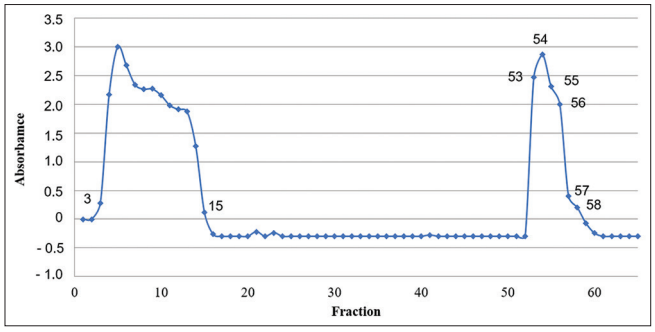

Figure 11: Graph of purification streptavidin recombination

CONCLUSION

The MBP fusion to monomeric recombinant streptavidin expression was successful in enhancing the solubility of streptavidin protein produced by *E. coli* BL21 (DE3) and can be purified using amylose resin.

Acknowledgment

We thanked to DIKTI and Padjadjaran University" was confirmed to be replaced with "Authors acknowledge the supports from Directorate General of Higher Education of Indonesia (DIKTI) and Padjadjaran University.

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Nil.

Conflicts of interest

There are no conflicts of interest.

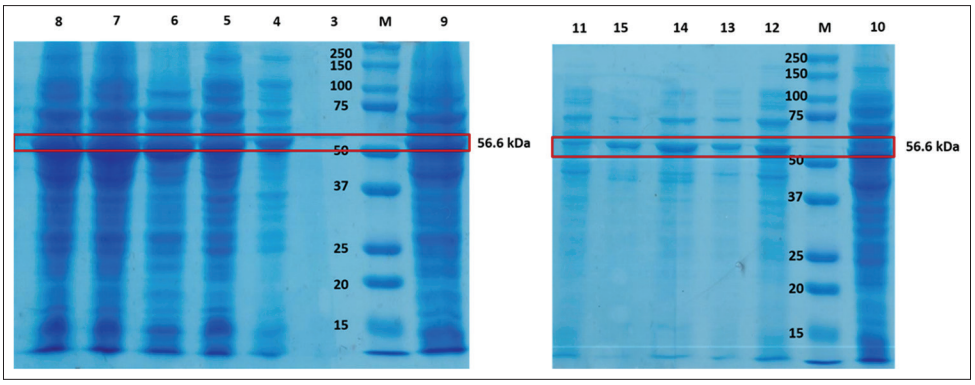

Figure 12: SDS-PAGE characterization of fraction 3rd-15th

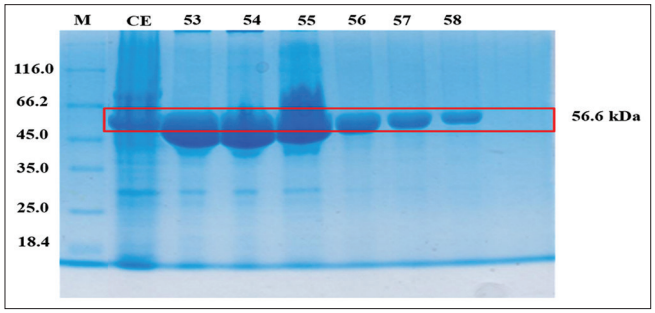

Figure 13: SDS-PAGE Characterization 53th-58th fraction. Notes: M = Marker, CE = Crude Extract

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