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RESEARCH ARTICLE

MOLECULAR WEIGHT DETERMINATION OF TOXIN PROTEIN FROM *E. COLI* ISOLATE PE16

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ABSTRACT

E. coli isolate pe16, when subjected to PCR analysis showed positive for*Stx2f*. Protein from this isolate was subjected to purification. The purification scheme involved ammonium sulphate precipitation at 40% saturation and then at 60% saturation, dialysis using 1% sucrose solution followed by Ion exchange chromatography. DEAE Cellulose for anion and CMC for cation were used as packing materials. Elutions obtained from above purification steps were subjected to protein estimation by Lowry's method. Crude and purified samples were analyzed by SDS-PAGE using molecular weight markers, to determine molecular weight and purity of the samples. It was observed that crude sample showed more of bands compared to ammonium sulphate precipitation and dialysis. From SDS-PAGE the purity of isolate confirmed a band with 7 kDa protein.

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INTRODUCTION

*Escherichia coli*is a gram negative, facultative anaerobic rod shaped bacteria found in lower intestine of human and animals that grows at pH 7, temp 37ºC. The toxins produced by *E. coli* strains include heat-labile and heat-stable enterotoxin causing lower fever, nausea, vomiting, stomach cramps and bloody diarrhea. Isolate pe16 which showed postive to toxins *Stx2f* 150bp, 7kDa and was subjected to protein purification method. The first step in purifying protein is preparation of crude extract. The extract contains a complex mixture of all the proteins. The debris is removed by ammonium sulphate precipitation, dialysis followed by ion-exchange chromatography. Sodium dodedyl sulphate polyacrylamide gel electrophesis–SDS-PAGE is useful for molecular weight analysis of proteins. The SDS detergent binds to the polypeptides to form complexes with consent charge to mass ratio. Molecular weights are determined by simulaneously running marker proteins of known molecular weight. Crude sample showed more of bands compared to ammonium sulphate precipitation and dialysis, after ion-exchange chromatography sample showed a clear band at 7kDa. Analysis of the purified toxins by SDS-PAGE revealed single protein band.

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MATERIALS AND METHODS

E.coli isolate

Isolate pe16 which showed postive to *Stx2f* toxin by PCR analysis was subjected to purification by ion exchange chromatography.

Purification of protein

E. coli culture was inoculated in 150 ml of nutrient broth and incubated overnight. Then culture was centrifuged at 10,000 rpm for 15 mins. Supernatant was subjected to ammonium sulphate precipitation (Rajan and Christy, 2010).To the supernatant of crude extract solid ammonium sulphate was added to 40% saturation and mixture was stirred at 4ºC for 2 h and centrifuged at 25,000 rpm for 30 min at 4ºC. The supernatant was adjusted to 60% saturation and the mixture was strirred at 4ºC for 2 hr and centrifuged at 25,000 rpm for 30 min at 4ºC. The precipitate was collected by centrifugation as described above and was dissolved in 20 ml of Phosphate buffer saline. Samples were then subjected to dialysis. Dialysis tubes, which were previously soaked in 1 M Tris HCl buffer, were used for dialysis of the precipitate. Sample was kept for dialysis in 1% sucrose at 4ºC for 18 h. (Rajan and Christy, 2010). Partially purified samples from above methods and elution from ion-exchange chromatography were subjected to SDS-PAGE.

SDS-PAGE (polyacrylamide gel electrophoresis)

500µl of partially purified protein sample from above steps of purification, crude preparation and elution from ion-exchange chromatography and low-protein molecular-weight markers were mixed with 5µl of dissociating buffer (0.5 Tris hydrochloride (pH 6.8) 12% sodium dodecyl sulphate (SDS), 5.0% 2-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue) and heated at 100ºC for 10min. 10µl samples were loaded onto individual lanes of 4% stacking gels (0.75 mm thick) and after electrophoretic run at a constant 150 v until the stacking dye reaches the top of the resolving gel (15%) and then at 200v until the dye front reaches the bottom of the resolving gel. Gel was fixed with fixing solution and stained with Coomassie brilliant blue for 3 hrs. Gel was destained using methanol-water containing 10% acetic acid for overnight. Finally, the gel was observed in uv illuminator for bands. A blue band on gel indicated the presence of protein (Laemmil, 1970).

RESULTS

Isolate pe16 which showed postive to *Stx2f* toxin was subjected to present study. Ammonium sulphate precipitation was used as initial purification and concentration step. Protein contents of sample from different steps of purification and the elution which showed more of protein concentration after ion-exchange chromatography was analysed by 12% SDS-PAGE using standarded molecular marker. When the electrophoresis was complete, the proteins in the gel were visualized by staining them with Coomassie blue. It was observed that after the sample was subjected to SDS-PAGE crude sample showed more number of bands compared to ammonium sulphate precipitation and dialysis samples (Figure 1). This indicates that there were some impurities. After purification by ion-exchange chromatography sample showed a single band (Figure 2). This confirms the purity of sample and improvement in purification process.

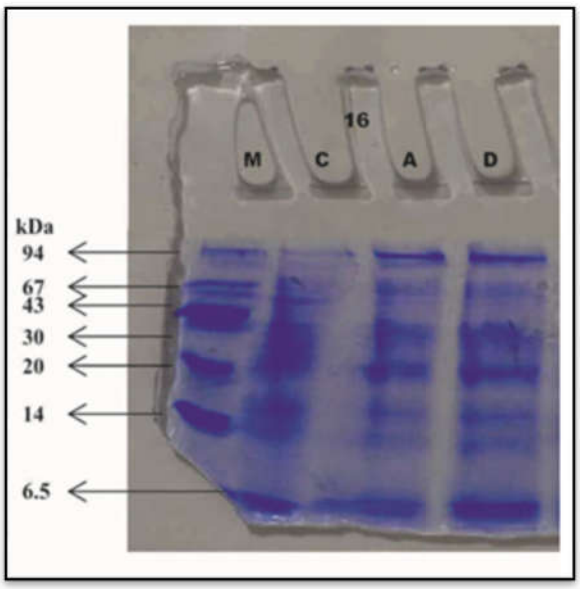

Figure 1. SDS-PAGE of protein from *E. coli* isolate pe16

M-Molecular weight marker, **C**-Crude, **A**-Ammonium Sulphate precipitation, **D**-dialysis

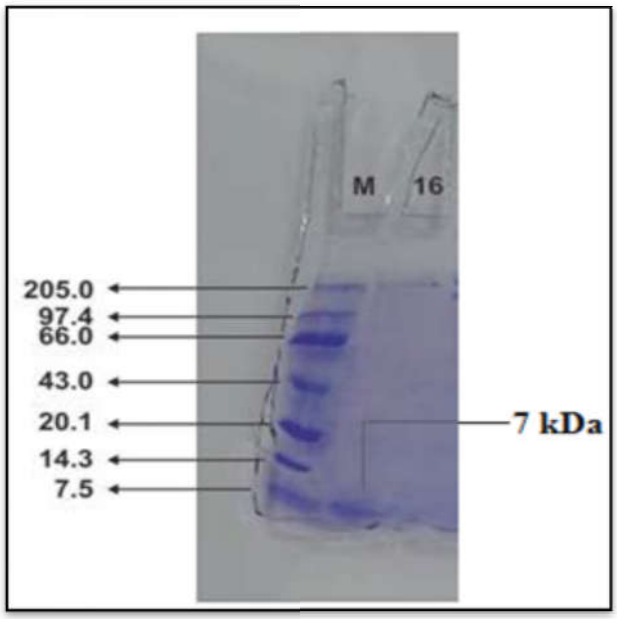

Figure 2. SDS-PAGE of protein from *E. coli* isolate pe16 M-molecular weight, Lane 16- purified protein from ion-exchange chromatography

DISCUSSION

Small proteins move rapidly through the gel, whereas large proteins stay at the top, near the point of application of the sample. SDS-PAGE is rapid, sensitive and capable of a high degree of resolution. As little as 0.1 µg of a protein gives a distinct band when stained with Coomassie blue. It was observed that in each step of purification purity of sample was seen as the bands were lesser. Efficacy of purification scheme can be analyzed by subjecting a part of each fraction to SDS-PAGE. The initial fractions displayed more number of proteins. As the purification steps progressed, the number of bands had diminished and the prominence of one of the band was seen. Brein'O *et al*. (1983) has carried out purification of *Shigella dysenteriae* I-like toxin from *E. coli* H30.

Conclusion

It was noticed that multiple bands were seen in ammonium sulphate precipitation compared to other steps of purification. As the sample was subjected to further purification bands was lesser and purity of sample was seen. SDS-PAGE is an easy method to identify the purity of sample with smaller quantity of sample.

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