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# Purification of toxin protein from *E. coli* isolate pe88

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## Abstract

*Escherichia coli isolate pe88, which showed positive to Stx2c toxin by PCR analysis was selected for the present study. The protein from E. coli culture was subjected to salting out by ammonium sulphate precipitation till the saturation followed by dialysis using 1% sucrose solution for overnight. Further purification of protein from the isolate was carried out by ion-exchange chromatography. Column was developed using DEAE Cellulose for anion and Sephrose for cation exchanger. Elution buffer was used as mobile phase. Procedures were performed at room temperature. Elutions collected every five minutes were subjected to protein estimation by Lowry's method using Bovine Serum Albumin as standard. The elution of protein from the isolate pe88 was subjected to SDS-PAGE using standarded molecular weight marker. The sample showed a single band close to 74 kDa after purification by column chromatography.*

**Keywords:** *E. coli*, DEAE cellulose, Sephrose, Lowry's Method, SDS-PAGE.

## Introduction

*Escherichia coli* is a gram-negative bacterium colibaccilli generally found in large intestine and colon of animals. It is facultative, anaerobic which grows at a pH of 7 with $37^0$C for 24 hrs. *E. coli* inoculated on Eosin methylene blue (EMB) agar shows a good growth of dark blue black colonies with green metallic sheen. It causes watery diarrhea, Cramps abdominal pain, Nausea, Vomiting, bloody stool which leads to cells leaks $Na^+$, $K^+$, and water. The toxins produced by *E .coli* strains include heat-labile or heat-stable enterotoxins. They are a family of related toxins with two major groups, *Stx*1 and *Stx*2. These toxins are responsible for lethal bloody diarrhea causing haemolytic uremic syndrome in humans causing damage to endothelial cells resulting in anemia. Among the different chromatographic techniques Ion exchange chromatography is the most frequently used technique for separation and purification of proteins based on charges[1]. By increasing the ionic strength the elution can be achieved. In gel filtration the protein is purified on the basis of their molecular size[2]. Lowry's method for protein estimation involves reduction of the Folin-Ciocalteau reagent and oxidation of aromatic residues mainly tryptophan. Thus the intensity of color depends on the amount of the aromatic amino acids present and thus varies for different proteins. Most proteins estimation techniques use Bovine Serium Albumin (BSA) as standard protein because of its low cost, high purity and ready availability. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) is used to detect the molecular weight of proteins. The molecular weight of the protein can be determined by simultaneously running the protein marker of the known molecular weight. The present study aims at purifiying the protein toxins in *E. coli*.

## Materials and Methods

**Isolation of *E. coli*:** Isolation of pe88 was inoculated on using Eosin Methylene Blue (EMB) agar media and was incubated at $37^o$C with pH 7 for 24hr. Nucleated colonies with green metallic sheen on EMB were selected for further studies.

**Anion-exchange chromatography:** The dialyzed pooled material 15mg of protein was applied to 13mm radius 150mm length column filled with DEAE cellulose A-50. The column was washed with elution buffer. The elution consists of five different concentration of Tris HCl and Sodium Chloride solutions i.e., 1M of NaCl solution and 1M Tris HCl buffer-pH 7.0. Elution of protein was monitored at 660nm[3].

**Cation-exchange chromatography:** The pooled material from anion-exchange chromatography was applied to 13mm radius, 150mm length column filled with CM Cellulose. The column was washed with elution buffer. The elution consists of five different concentration of Tris HCl and Sodium Chloride solutions i.e., 1 M of NaCl solution and 1M Tris HCl buffer-pH 7.0 (3). Elution of protein was monitored at 660nm. Protein estimation was performed to know the purified elution by Lowry's method and using standard BSA curve mg/ml of protein was calculated.

**Gel-filtration:** The column was filled with Gel bead matrix (Sephadex G75) measuring about 13mm radius and 150mm length. Flow rate was set to 1ml/5min.Sample was added after the matrix settled.100mM Phosphate Buffer (pH – 7.0) was used to stabilize protein (mainly enzyme). Elutes were collected every 5 minutes. The range of elution was from 20 – 50 depending upon purification needed. Protein estimation was

performed by Lowry's method[4] using standard BSA to know the purified molecules in which 10µl of eluted sample along with 2.5ml of water, whose absorbance was read at 280nm.

**SDS-PAGE:** 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. 10µlsamples 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[5].

## Results and Discussion

Isolate pe88 showed nucleated colonies with green metallic sheen on EMB agar (Figure-1), isolate which showed to *Stx2c* toxin by PCR analysis was selected for the 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 by ion- exchange were analyzed by 12% SDS-PAGE with standarded molecular marker. It was observed that after sample subjected to SDS-PAGE crude sample showed more number of bands compared to ammonium sulphate precipitation and dialysis samples (Figure-2).

This shows that there were some impurities. The fraction with highest protein concentration eluted (Table-1) was subjected for SDS-PAGE using standard molecular weight after purification by ion-exchange chromatography sample showed a single band (Figure-3). This confirms the purity of sample and improvement in purification process. Protein of interest showed a prominent band compared to other bands in partially purified samples. Movement of small proteins thru the gel is rapid as compared to the large proteinssample. As little as 0.1 µg of a protein gives a distinct band when stained with Coomassie blue. The initial fractions displayed dozens to hundreds of proteins. As the purification steps progress, the number of bands had diminished and the prominence of one of the band was seen. Proteins that have a low density of net positive charge will tend to emerge first, followed by those having a higher charge density. The Ion exchange chromatography showed less recovery of protein compared to Gel Filtration Chromatography Brein and Laveck[6] have carried out purification of *Shigella dysenteriae* I-like toxin from *E. coli* H30.

**Figure-1**
**Growth of *E. coli* on Eosin Methylene Blue agar medium**

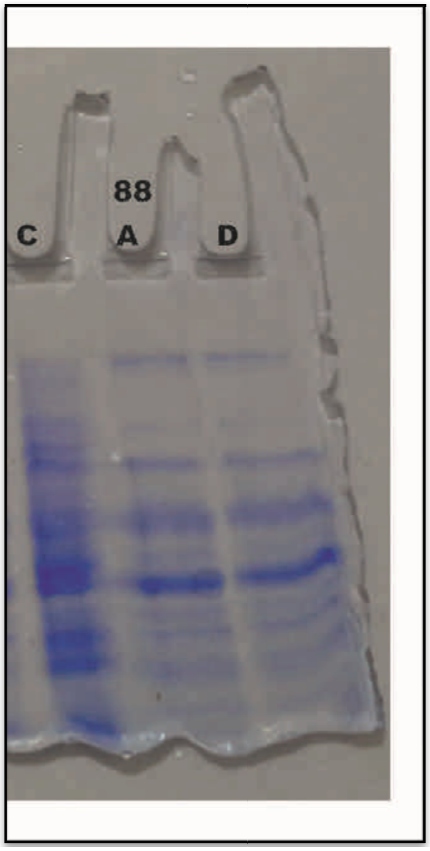


**Figure-2**
**SDS-PAGE of protein from *E. coli* isolatepe88 C-crude, A-ammonium sulphate precipitation, D- dialysis**

**Table-1**
**Concentration of Protein in different elutions from isolate pe88**

| Elution no | Volume of BSA (ml) | Volume of distilled water (ml) | Volume of copper reagent (ml) | Incubate for 10mins at room temperature | Folin reagent (ml) | Incubate for 30mins at room temperature | OD at 660nm | Mg/ml |
|---|---|---|---|---|---|---|---|---|
| 3 | 0.1 | 0.9 | 5 | | 1 | | 0.100(anion) | 1.140 |
| 5 | 0.1 | 0.9 | 5 | | 1 | | 0.281(cation) | 3.211 |
| **Gel filtration** | | | | | | | **OD at 660nm** | |
| 6 | 0.1 | 2.5 | 5 | | 1 | | 0.009 (anion) | 1.028 |
| 9 | 0.1 | 2.5 | 5 | | 1 | | 0.022 (cation) | 2.51 |

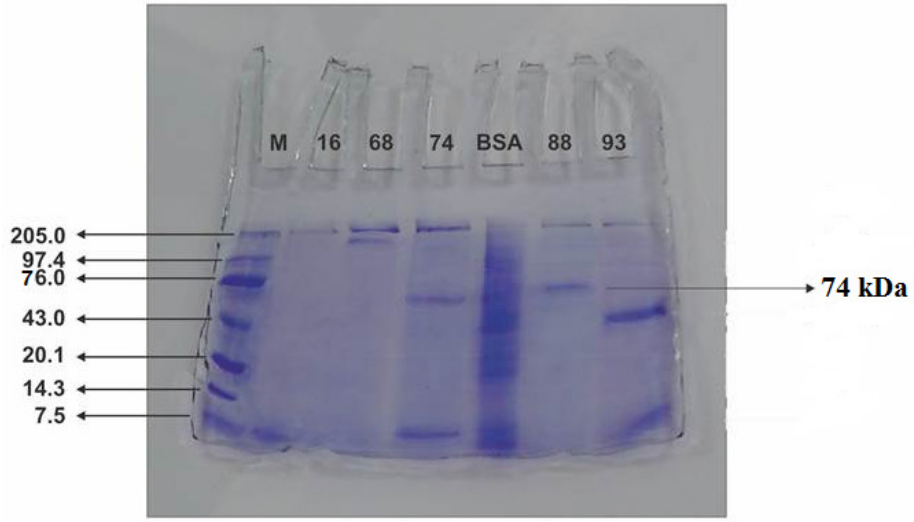


**Figure-3**
**SDS-PAGE of protein from*E. coli* isolate pe88 M-molecular weight, BSA-Bovine Serum Albumin**

## Conclusion

It was observed that in each step of purification purity of sample was seen, as the bands were lesser. A part of each fraction of purification steps can be examined by SDS-PAGE. SDS-PAGE is a sensitive method with a high degree of resolution.

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