Clinical application Protease from *Acinetobacteria* Species.

Salunke.S M[1*], Dr. Jadhav B. N[2], Dr. Satpute. R. A.[3]

[1*]Department of Biotechnology, Vinayakrao Patil Mahavidyalaya, Art's Commerce and Science College Vaijapur. Chhatrapati Sambhajinagar-423701, Maharashtra, India.
[2]Department of Zoology, Vinayakrao Patil Mahavidyalaya, Art's Commerce and Science College Vaijapur. Chhatrapati Sambhajinagar-423701, Maharashtra, India.
[3]Government Institute of Science, Department of Botany Chhatrapati Sambhajinagar-423701, Maharashtra, India.

***Corresponding Author:** Salunke.S M
sweetysalunke25@gmail.com.

Abstract:- Extracellular protease from *Acinetobacteria* species isolated from fish gut. The protease was produced and purified by ammonium persulphate, dialysis, Sephadex G column chromatography and Ion Exchange Chromatography and HPLC. Characterization was done by using BAPNA substrate and MALDI-TOF. Purified protease was studied for anti- inflammatory and anti-cancerous activity. The 50 percent inhibitory concentration was as followed for IL-6 0.348 mg/ml and LNCAP cell line 30.11µg/ml.

Keywords:- Protease, *Acinetobacteria* species and anti-inflammatory and anti- cancerous activity.

Introduction:-

Protease are the important class of enzymes can be extracted by microbial source for large scale production. *Acinetobacteria* species are gram negative bacteria belong to class gammaproteobacteria and family Moraxellaceae [2]. It has ability to produce extracellular protease like serine protease S1A trypsin [5]. The extracellular protease can be easily recovered by downstream process during batch fermentation [8]. The trypsin plays important in inflammation and cancerous activity. It makes the macrophages and monocytes active and move at the site of infection [10,11]. Trypsin role in gelatin degradation at the time of fertilization, it helps the sperm to swim [3]. It has diverse role in different industry like dehairing of hair in leather industry, destaining in detergent, eye lenes cleaning, silver recovery from X-ray film [1,4,6,7,9].

In the present study the extracellular protease was purified and studied for clinical application against IL-6 by ELISA and LNCAP cell line of prostate gland by MTT assay.

Material and Methods:-

Pure culture of *Acinetobacteria* species.

Inoculum Preparation

A loopful suspension *Acinetobacteria* species of was inoculated in 20ml Nutrient broth.

Production Media

The Batch culture media was prepared by adding in 250 ml of Yeast extract 3gm, Maltose 3gm, $MgSO_4$ 0.5, K_2HPO_4, 0.5, $CaCl_2$, 0.2, KCl 0.2, $NaCO_3$ 0.5% was autoclave at 121ºC for 15- 20min. After media cool down was transferred to laminar air flow and 5ml overnight grown culture was inoculated.

Enzyme purification

Precipitation by 40-60% w/w Ammonium persulphate, dialysis membrane preparation by 2% sodium bicarbonate and EDTA and boil for 15-20 min, pack the one end of dialysis with thread add precipitated protease then again seal the other end, suspend the dialysis bag in the Tris buffer HCl 20mM overnight, Tris Buffer gel Chromatography glass column chromatography 2×50ml, stationary phase Sephadex-G 25 mobile phase 25mM Tris Tris buffer. Ion exchange Chromatography anion exchanger Resin DEAE cellulose and elution buffer 0.1- 1.0mM. HPLC, Characterization by BAPNA substrate and MALDI-TOF. The anti-inflammatory and anti-cancerous activity was performed by Progenome Life Science.

Protein content was estimated by Lowry *et al* method using bovine serum albumin (BSA) as the standard at 660nm.

Protease activity by Anson method, the activity was measured against the release of tyrosineµg/min at 660nm using 0.5% of casein as substrate in 50mM glycine NaOH of pH 9. The reaction was terminated by adding 10% of TCA trichloroacetic acid of 0.5 ml.

Trypsin activity amidase activity was measured by using BAPNA (Na-benzoyl-DL-arginine- p-nitroanilide hydrochloride) as substrate. BAPNA was prepared by adding 43.5 mg of BAPNA in 1 ml DMSO solution and adjusting volume 50mM Tris-HCl containing 10mM $CaCl_2$ pH-8. The 0.5ml protease was used by adding 0.5 ml of BAPNA substrate incubate for 20 min. Then followed by reading at 410nm. The one unit of enzymes release 1µmol of nitroaniline

from BAPNA/ml/min.

Results:-

The overnight grown culture 5ml was inoculated in 250ml of production media and incubated in orbital shaker at 120-150rpm at 30-40ºC. After 24 -48 hours the extracellular protease by collecting filtrated the filtrate was used as crude protease the protein and protease activity was measured by Lowry's and Anson method. Purification starts from subjecting the filtrate to generate cell free medium by centrifugation at 5000 rpm at 5ºC and the supernatant was collected. The collected samples were further for precipitation by salt ammonium per sulphate, then molecular size separation by gel filtration and depending upon charge Ion-Exchange chromatography, after each step protein and protease activity was measured, HPLC shows two peak of trypsin as compared with standard trypsin and then SDS- PAGE to determine molecular weight was in between 23-25 KD Characterization was done by using BAPNA trypsin activity was 10U. HPLC collected fraction used for MALDI-TOF it shows 51% percentage score of matching HPLC fraction was further used to check the anti-inflammatory and anti- cancerous activity. The clinical application of purified protease anti-inflammatory activity against IL-6 was 0.348mg/ml and anti-cancerous activity against LNCAP prostate gland cell line was 30.11µg/ml.

Table No:1 Protease Activity Assay

Sr.No	Purification steps	Total Protein in mg/ml	Total Protease U/ml	Specific Activity U/mg
1	Crude Enzyme	58	170	2.9
2	Ammonium Per Sulphate	20	98	4.9
3	Dialysis	12	75	6.2
4	Sephadex G-25	8	54	6.7
5	Ion Exchange Chromatography	2	17	8.5

Fig No:1 Acinetobacter Species on Skim Milk Agar

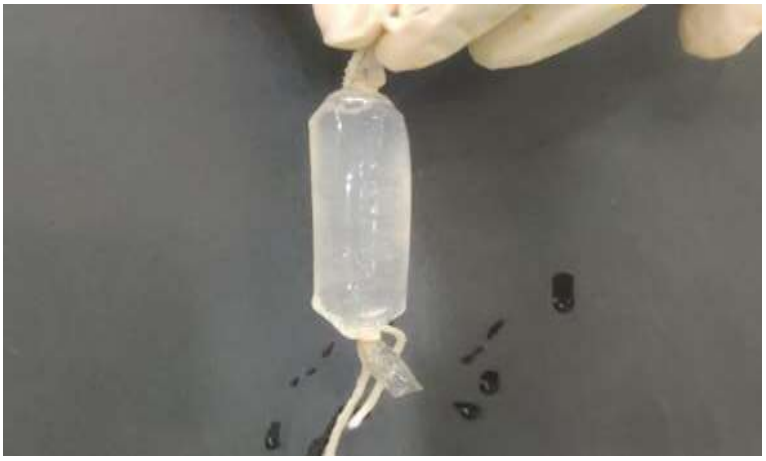

Fig No: 2 Dialysis of Protease.

Fig No 3: Sephadex Gel Column Chromatography.

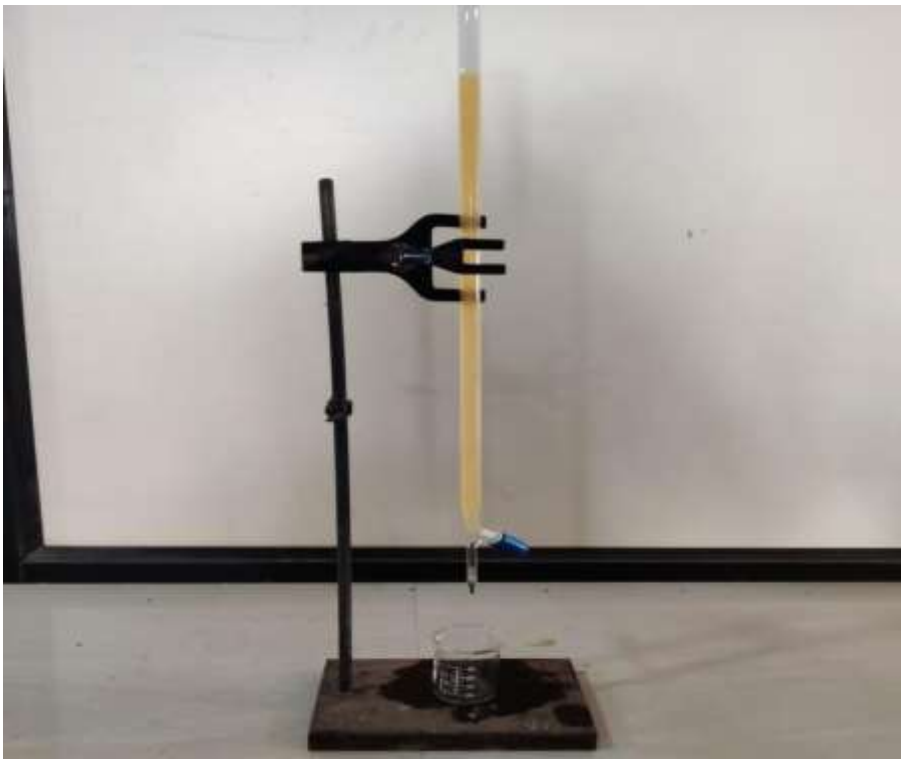

Fig No:4 Ion exchange Chromatography.

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