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RESEARCH ARTICLE

SIMPLE RELEASE OF PROTEIN A FROM STAPHYLOCOCCUS AUREUS NCTC 8325

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ABSTRACT

Protein A is found in the cell wall of bacterium *Staphylococcus aureus*. It can be used in the purification process of monoclonal and polyclonal antibodies. Enzymatic method using Lysostaphin Enzyme is the most common and standard method for the isolation of protein A. Never the less this method is very expensive in an industrial Scale. Hereby, we have developed an alternative method to isolate this protein without Lysostaphin digestion, in a time and cost effective manner. Five ways Hot Acid, Freeze and Thaw, Toluene- Triton× 100 – EDTA, Lysozyme and Sodium Meta Priodate were used to lysis of cell wall bacteria were performed. Here in this study we compared five mentioned method to achieve bacterial cell wall lysis. The obtained results were compared to the standard Lysostaphin digestion method. In all of the studies *Staphylococcus aureus* strain NCTC *8325* was used for protein A extraction. Bacterial cell wall *53* KD a protein A, extracted from the mentioned strain, was purified by ammonium sulfate (*80*%) precipitation method. The results were analyzed and confirmed via Gram staining for microscopic evaluations, SDS-PAGE Analysis, Validated Lowry assay, ELISA Test with both human and goat serum samples, and western blotting with rabbit IgG. The obtained data were compared to assess the yield of protein extraction. The results revealed that *300*µg protein A per 300g wet weigh of Cowan strain was extracted via lysozyme digestion method, compared to the developed method, 283.7 µg protein A per 50g wet weigh of the same strain. This amount approximately 6 fold increased which is statistically significant. According to the obtained results stated above, we can conclude that utilization of lysozyme digestion method following ammonium sulfate *80%*, Precipitation method is and effective method in the terms of cost and ease of industrial production line design.

Key Words: Approximately, Increased, Statistically significant, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcal protein A (SpA) plays an important role in molecular biology owing to its specific interaction with the Fc portion of immune globulins from many mammals. Biological responses to SpA include activation of the complement system, hypersensitivity reactions, cell-mediated cytotoxicity, interferon production, activation of polyclonal antibody synthesis and mitogenic stimulation of lymphocytes many immunological methods have been developed and refined using SpA as a reagent, including immune precipitation techniques and double sandwich immune assays. In addition, solid-phase protein A has been used therapeutically to decrease the amount of circulating immune complexes in sera Treatments involving continuous-flow extra corporal systems have been tried on patients with advanced carcinomas and acquired immune deficient syndrome (AIDS).Because of its importance as an immunological tool, extensive structural and biochemical studies of the protein A molecule have been performed during the last two decades Fragmentation of the SpA molecule by trypsin digestion followed by purification and amino acids sequence analysis of the fragments suggested a tetrameric structure of the IgG-binding. Protein A is a commercial product that is economically important and expensive with so many advantages. The other applications from protein A, can be referred to monoclonal antibody purification where the antibodies found with high importance in treatment of malignancies, treatment of inflammatory mediators and treatment of allergic diseases. According to the studies provided to date, in Iran just little studies have been conducted on Staphylococcus aureus protein A, where expression and purification of protein A from cell walls of bacteria as a commercial product with the value for IgG purification and the economic usage found of importance, reporting no study on this topic has been provided yet. Preparation and purification of Staphylococcus aureus subsp. aureus (strain NCTC 8325) protein A and getting used it in Purification of immunoglobulin G, extraction of protein A, with use of proper methods and then sticking it into a proper bed for the purification of IgG reported as the major goals of this study.

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Protein A

Protein A is a protein in the cell wall of *Staphylococcus aureus* made of a *53 kDa* in *NCTC 8325, and developed 1.7% of the Cell weight with the total 450 amino acid. 4 tyrosine units which fully exposed on the cell surface of bacteria are responsible for biological activity. This protein can be isolated using permeation chromatography and Lysostaphin digestion on IgG sepharose*. In some strains of *Staphylococcus aureus,* such *proteins are found to form Secretory and extracellular aggregates.* Protein A isolated for the first time in 1972 from cell walls of Staphylococcus aureus. Ankarest and colleagues in 1976 recognized more than 95% IgG serum join to Staphylococcus aureus protein A. Seven lofthel *et al*. (1982)

cloned protein A gene into Escherichia coli PbR322. Mota and Guss (1982) sought to extract Protein A using bacterial mutant strains in culture which used to have extracellular protein production and secretion. Sting *et al*. (1990) sought to extract Protein A from cell walls of bacteria. Warnes *et al*. (1993) during the process of cloning this protein, observed that expressing this protein in total in Escherichia coli bacteria leads this protein aggregates in bacteria membranes whereby it would be avoided from Septa development where if the gene in membrane removes, there would be no matter. Frenay and his associates (1994) proposed a useful and fast method to determine typing of Staphylococcus aureus resistant to methicillin using the sequence of Staphylococcus aureus protein A which was determined previously. Hariss *et al*. (2002) conducted several studies on the cell wall of Staphylococcus aureus and how to extract the proteins. Hober *et al*. (2006) addressed purification of antibodies, IgG class, using column chromatography and protein A.

Kobayesh and colleagues (2009) showed the gene existing in this protein in different geographic areas contain Polymorphism where based on the Polymorphism in X gene of this bacteria, 42 clinical strains isolated and this point emphasized the impact of Protein A in outbreak of this bacteria. Bahram Kazemi and his colleagues (2009) provided Staphylococcus aureus protein a cloning in Expression plasmid with the use of Affinity chromatography. Garofalo *et al*. (2011) investigated the role of Spa gene and protein A in induction of inflammation and osteomyelitis-consequently recognized a direct correlation between this protein and pathogenicity. Watanab and his colleagues (2013) recognized that optimization of protein A purification to cope with bottleneck in the process of high-cost production to treat antibody is of importance.

MATERIALS AND METHODS

Preparation of Bacterial strains and its culture

Staphylococcus aureus strains lyophilized and provided from Pasteur Institute of Iran, Department of bacterial vaccines and antigen preparation with number NCTC8325 and then cultured in Brain-heart infusion Agar (Himedia) then culture prepared from bacterial incubated at 37 ° C for 24 hours.

Methods of extraction

Hot Acid

Suspension Bacteria prepares in 40 ml PBS at pH 2 they were heated for 10 min at 95°C in a water bath.

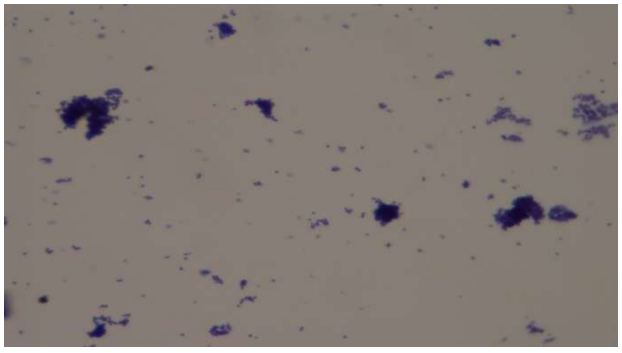


Fig.1. Microscopic slides of Triton lyses method for the cell wall of Hot Acid

The suspension was subsequently neutralized with 0.5 molll NaOH and the bacteria removed by centrifugation (20 min, 13000 X g) And the solution on the surface passes through filter with the Pore around diameter of 45/0 mm, and Protein measurement using Lowry method was performed on the supernatant.

Toluene /Triton X-100, (EDTA)

50 ml Tris-Hcl 0.02 M, PH7.5 with 2.5 ml of toluene,0.025 micro liter Triton X100 and 0.05 mg of EDTA added to 5 g of Cellular weight, and put for 30 minutes in shaking incubator at 37 ° C, centrifuged at 3500 × g round for 20 min so that Protein measurement using Lowry method was performed on the supernatant.

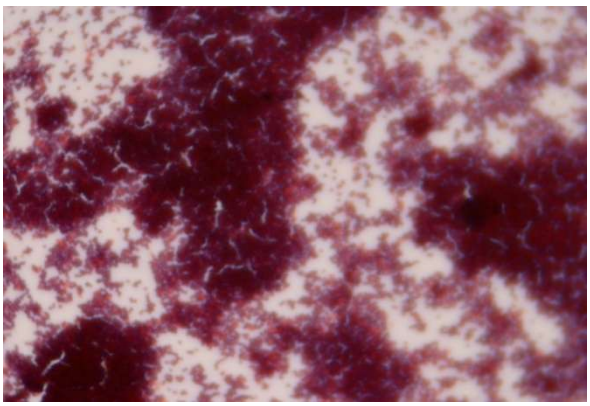
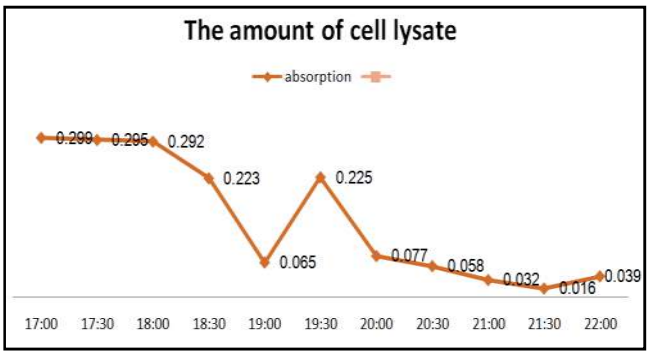
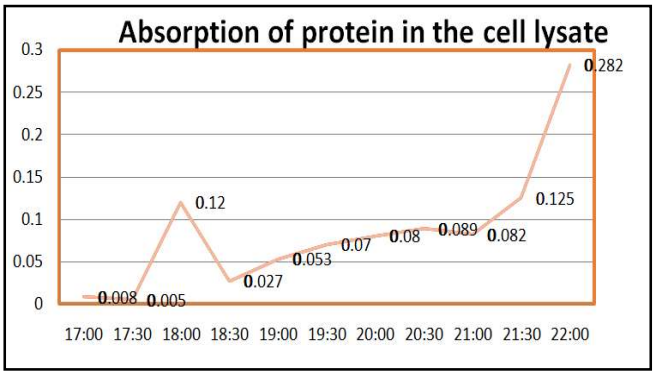


Fig. 2. Microscopic slides of Triton lyses method for the cell wall of toluene, Triton and EDTA

Freez and thaw

This includes two stages of cellular Lysis and Freez and thaw. 5 g of cellular weight suspended with 50 ml of cold water, 50 ml of 0.5 M Tris base and 05 0.0% Triton, put in shaking incubator at 30 ° C, where read during 5 hours every 30 minute in wavelength of 580nm.





Decrease at absorption in the last time interval displayed Lysis. At second stage, Suspension put in the freezer for -12 ° C for 12 hours and then carried out at labrotorary and vortex temperature and then centrifuged at 1300 × g round so that Protein measurement using Lowry method was performed on the supernatant.

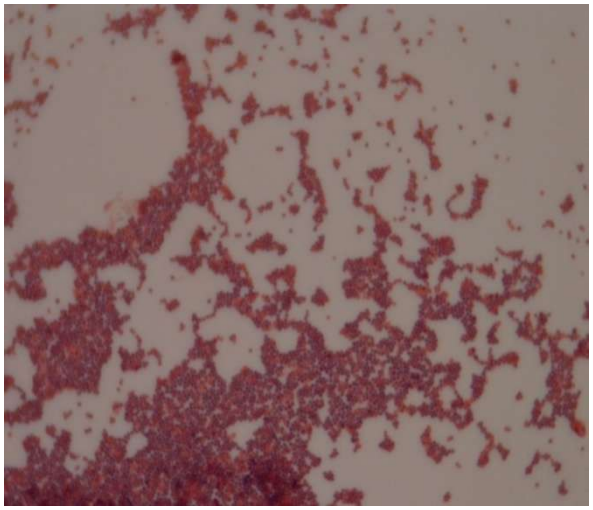


Fig.3. Microscopic slides of Cellular lysis of Freez and Thaw method

Lysozyme extraction

Suspension prepares for 20 ml Phosphate-buffered Saline with 0/0015 mol/l PMSF, 50mg sodium azide and 50 mg lysozyme. This suspension is placed in shaking incubator at 37 ° C for 22 hours. Then bacteria centrifuged at 13000 round where on bacteria are deposited. And the solution on the surface passes through filter with the Pore around diameter of 45/0 mm, and Protein measurement using Lowry method was performed on the supernatant. To determine protein with Lowry method.

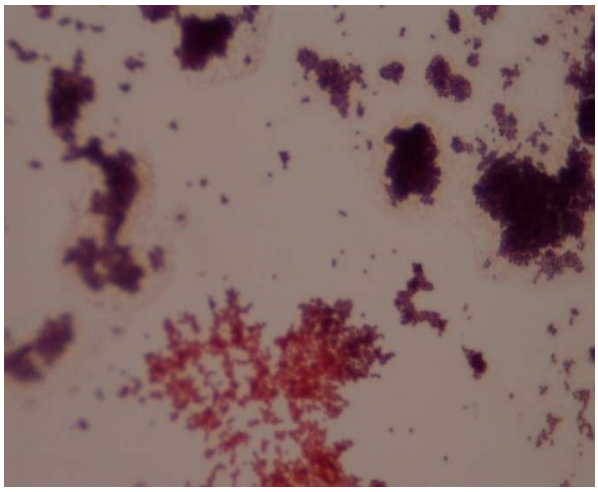


Fig. 4. Lyses method of cell walls using Lysozyme method

Extraction of Sodium metaperiodate

Bacteria with 40 ml of 0.1 ml/l Sodium metaperiodate solution with the content of mol/l PMSF0/0015 and mg 100 sodium azide suspended where centrifuged at 13000 round for 20 minutes and the solution on the surface appeared with Hemodialysis against PBS for 24 hours at 5 ° C, and Protein measurement using Lowry method was performed on the supernatant.

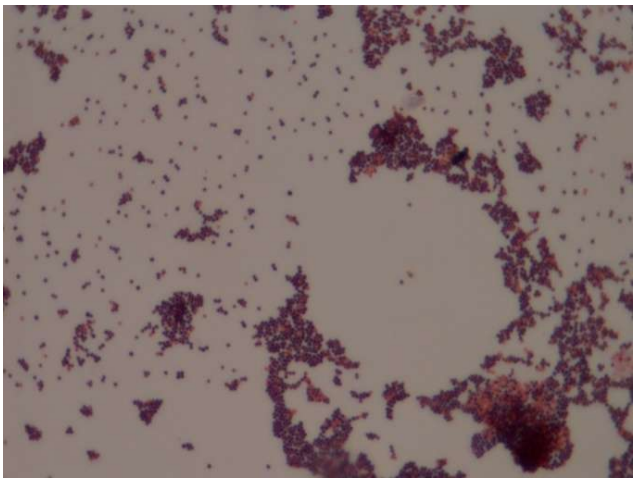


Fig.5. Microscopic slides of Cellular lyses method with Sodium metaperiodate

Protein A could be Isolated from strain NCTC 8325 of Staphylococcus aureus by lysozyme, hot acid, freeze and thaw, sodium Meta priodate and toluene - triton X100-EDTA and was purified by ammonium sulfate (*80*%) precipitation method. Methods are compared with Protein measurement, Gram staining and microscopic observation, SDS-PAGE, Elisa and Western blot.

IgG Binding protein a with Elisa Method

The micro plate 96 was used so that the phosphate buffered saline was poured micro liter of all homes in 1000and then to 100micro litres of protein A to the first two rows and add rows to the end of the serial dilutions of protein A produced 1.2 was Duplicate dilutions are made and the micro plate at room temperature for 24hours was called to the stage coating. normal human serum, phosphate buffered saline was diluted 1: 100 and 100 microliter plate in row A Shadow cast by the end of the rows of serial dilutions were prepared at a concentration of 2.1 except for row H was poured in which goat serum . microplate for 30 min at 37 ° C was incubated. Finally, all wells of the plate absorbance at 450 nm were measured by ELISA reader.

RESULTS AND CONCLUSION

using the obtained findings were shown that all procedures carried out to be able to break the walls of bacteria and protein rates slipping A note to be released but it should be noted that the maximum amount of proteins related to the lysozyme method.

Protein measurement

Lowry method was performed on the supernatant in Hot Acid were 1.65 mg per 0.5 ml. For toluene, Triton X100 and EDTA where found 18.23 mg per 0.5 ml. To determine protein in Freez and Thaw 108.94 mg per 0.5 ml. And for Lysozyme method where found 141.85 mg per 0.5 ml. in sodium meta priodate method were 39.85 mg per 0.5 ml.

SDS-page

Five samples placed in wells where five wells associated to 5 methods of lysis of bacteria to extract purify Staphylococcus aureus protein A. Staphylococcus aureus subsp. aureus (strain NCTC 8325). Finaly Sodium metaperiodate, lysozyme and

Freez and Thaw method had obvious band but methods of hot acid and Triton lacked protein and they have Unclear and unspecified band.

noted that the column allows purification of polyclonal and monoclonal immunoglobulins with high purity to be easy, fast and efficient causes.

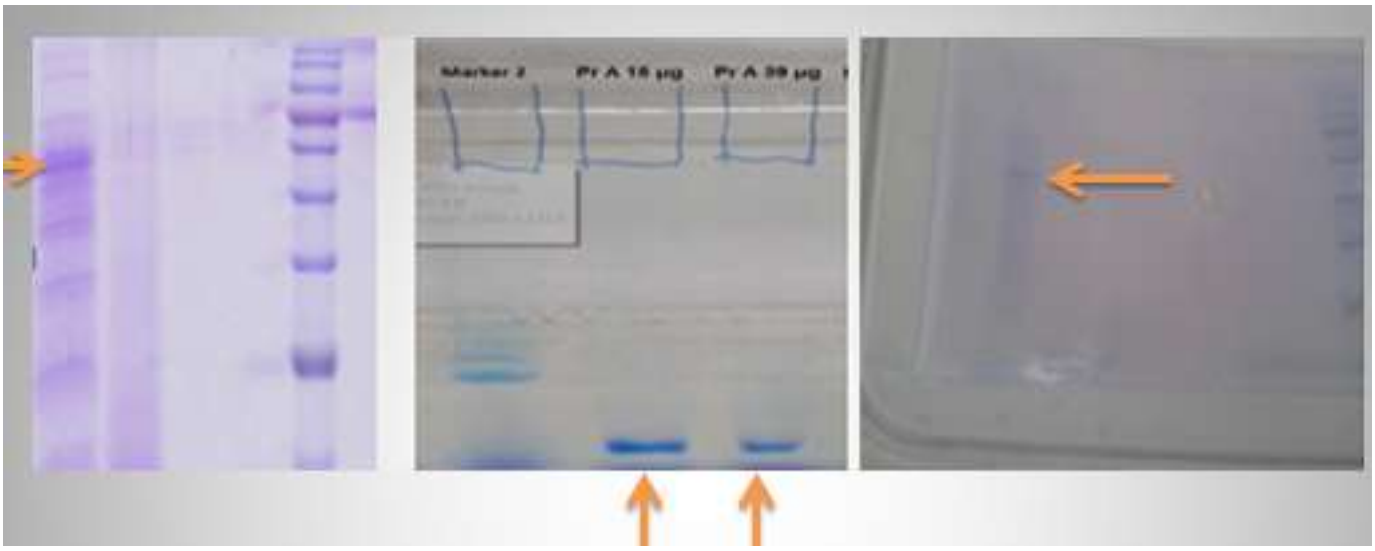


Fig.6. Protein electrophoresis

Elisa

Protein A were extracted and purified carried out for all the methods used where an investigation using ELISA reader showed that absorption in a dilution of 1/128 with human normal immunoglobulin G, which suggests a strong connection to human plasma protein A and the lowest absorption at H which indicates poor connection between protein A and serum of goats.

Western blot

The protein in the sample was in the range of 48 to 63 KD molecular weight protein equivalent of a level is 8325 NCTC.

Fig.7. Western blot

According to the obtained results stated above, we can conclude that utilization of lysozyme digestion method following ammonium sulfate *80%*, Precipitation method is and effective method in the terms of cost and ease of industrial production line design. To verify the obtained protein quality the purified protein A was utilized to prepare an affinity chromatography column. Matrix intended to purify rabbit serum IgG. To prepare affinity chromatography columns based on protein A is used for the separation of serum IgG should be

In many researches lysostaphin was used to lysis staphylococcus cell wall. In this research in order to extract and purificate we used lysosome and other ways. Our results indicate that this procedure is an alternate procedure and have economically advantage for purification. In this study it can be isolated without lysostaphin digestion, which makes the procedure rapid and inexpensive

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