Chapter 5

Conclusions

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Staphylococcus is a commonly encountered organism of the human body. Staphylocoagulase isolated from the culture filtrate of the strain is a simple protein. The clumping action of the protein using slide coagulase tests confirms the rapid plasma agglutination. Growth on MSA has been considered a valuable media for identification and characterization the isolates of *Staphylococcus* strains. Staphylocoagulase may have an important role in the homology of the coagulation cascade. It seems theoretically possible that coagulase may favor a hypercoagulable state in situations of simple blood loss, as in trauma, surgery, etc. Since coagulase can act in the presence of such anticoagulants as heparin and sodium citrate, it is conceivable that it may counteract excessive bleeding induced by such anticoagulants. Staphylocoagulase is a significant enzyme for the identification of *Staphylococcus aureus.*

In the present study disk diffusion test on agar plates was an excellent method to determine the antibiotic resistance of the specific strain and vice versa. One step extraction of the protein using acetone is nevertheless the best method found for collecting high amount of coagulase. High protein yields were found while estimating the protein from ammonium sulphate precipitation. The results of this study suggested that Staphylocoagulase isolated from the culture of the bacterial strain is a simple protein of three fragments with molecular weight of 66, 33, and 14 kDa. The formation of clotting of the blood sample is a combine action of these three fragments. The finding of this experiment is supported by the work of Kawabata *et al.* (1985). In the present study, these three fragments have been isolated, characterized, and tested with human blood samples. The purity could be demonstrated from the polyacrylamide gel analysis. This study also points towards the presence of a primary structure of a polypeptide chain.

The HPLC analysis of the gel filtrate showed the presence of three prominent amino acids. Our study showed the potentiality of the intrinsic mechanism of

coagulation initiated by Staphylocoagulase. But further investigations are required. Different types of results may come from other studies due to the use of different media and because of the totally different strain.

The results of clumping studies and molecular modelling demonstrate a novel mechanism of staphylothrombin recognition and cleavage by the conformationally activated fibrinogen. Homology modeling is the simple and most reliable approach as it is an extrapolation of protein structure for a target sequence using the known3D structure of similar sequence as a template. Based on the facts, that proteins with similar sequences are likely to assume same folding. Certain proteins with as low as 25% similarity have been observed to assume same 3D structure. The generated three-dimensional structure of staphylocoagulase in the present study will be helpful to understand the mechanism of the coagulase action. Furthermore, in this *in silico* characterization and structure prediction via homology modelling offers an alternative way to understand the nature of staphylocoagulase as well as structural information well before the structure of the protein is determined by other experimental techniques like X-ray crystallography and NMR. *Staphylococcus* is a commonly encountered organism of the human body, causing serious infections. But as per the structural analysis and toxicity predication done through the bioinformatic tools, we found that the staphylocoagulase, extracted from soil *Staphylococcus* strain, is non toxic. The findings of the experiment support the suggestion of Menken and Walston (1955) that the coagulase protein is not toxic.

The present findings indicate that coagulase from *Staphylococcus* is an extracellular protein that bound to the cell wall and is responsible for the slide test coagulation. The enzymatic properties of staphylothrombin are still left to be studied. Our assumption is that coagulase may favour a hypercoagulable state in cases of simple blood loss, as in trauma, surgery, etc. It is because of the effects on blood coagulation and other factors related to the coagulation cascade. Action of coagulase in the presence of anticoagulants such as heparin and sodium citrate was also recorded. The functional region of staphylocoagulase, which binds to human prothrombin and induces prothrombin activation, still needs further research. The present study shows

the potentiality of this intrinsic mechanism of coagulation initiated by staphylocoagulase which may have an important role in the homology of the coagulation cascade.