

CHAPTER 6

Conclusion

CONCLUSION

In conclusion, the current study reported the exploration of protease-producing gut microbiota from the *Monopterus couchia*. *Bacillus safensis*, isolated from the gut, demonstrated the highest proteolytic activity. The enzyme demonstrated antibacterial activity against pathogenic bacteria. Furthermore, it showed blood-stain removal property depicting its potent role in the cleaning process of the detergent industries. The protease was purified and characterized subsequently. The trypsin like serine protease gene of 909 bp (*knbs*^{SP1}) was isolated, cloned into PET-28a expression vector. The recombinant plasmid pET28a-KNBS^{SP1} was expressed in the competent cells of *E. coli* BL21 (DE3). The recombinant protease enzyme purified through Ni-NTA affinity chromatography and characterized subsequently. The primary, secondary, tertiary structures, and the protein-protein interactions of *B. safensis* serine protease was elucidated. The assessment of the secondary structure demonstrated predominance of coils with the increase in KNBS^{SP1} stability and activity under high temperature and alkaline conditions. Furthermore, docking of the modeled trypsin like serine protease showed good affinity towards casein substrate. Overall, the study referred to the cloning of the *knbs*^{SP1} gene from *B. safensis* that encodes trypsin like serine protease (KNBS^{SP1}) enzyme enriched with properties which are important for the laundry and leather industries. The rKNBS^{SP1} protease showed better activity and stability at high temperature (70 and 80°C) and pH (9 and 10) as compared to non-recombinant protease. Additionally, comparative analysis of the blood parameters of *Monopterus couchia* with human blood exhibited the haemoglobin value as well as other blood parameters of the *M. couchia* blood comparatively higher values than that of human blood. Therefore, raw blood of the fish could be prescribed for the treatment of acute anaemic patients, as prescribed by the traditional medicine-men.