CHAPTER 7

Summary and Future Work

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7.1. Summary

Noxious chemical processes in various industries like detergent and leather, causing severe environmental problems need to be replaced by eco-friendly alternatives. Accordingly, the microbial enzymes have gained enormous attention owing to their industrial applications. The current study reported the exploration of protease-producing gut microbiota from the unexplored freshwater Asiatic mud eel (*Monopterus cuchia*) for the first time to the best of our knowledge. *Bacillus safensis* PRN1 was isolated from the eel and pure cultured. The gut determined bacteria exhibited significant protease production. The *B. safensis* exhibited the optimized proteolytic activity and the bacterial growth at incubation time 72 h, pH8, temperature 40°C, carbon source fructose, and the nitrogen source gelatin. 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 of a 33 kDa was purified and characterized subsequently. The protease enzyme showed activity in a wide pH and temperature regions with an optimum at pH8 and temperature 60°C. The enzyme was found to be serine protease as it is strongly inhibited by PMSF. The protease was found to be stable towards various surfactants and detergents demonstrating its potential application in the detergent industry. 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 purification process demonstrated high purification yield and better fold that are proficient in industrial processes low-cost approaches. The recombinant protease demonstrated tolerance against high temperature and pH. It also displayed significant stability and compatibility towards inhibitors, metal ions, surfactants, oxidizing agents, and commercial detergents. The primary, secondary, tertiary structures, and the protein-protein interactions of *B. safensis* serine protease was elucidated. KNBS^{SP1} protease is classified as trypsin like serine protease on the basis of multiple sequence alignment. 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 cuchia* with human blood exhibited the haemoglobin value as well as other blood parameters of the *M. cuchia* 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.

7.2. Future work

In-depth study of cuchia blood in relation to the human blood has to be investigated for establishing its therapeutic use in the treatment of acute anaemic patients as prescribed by the traditional medicine-men. Synthesis of nanoproteases and their assessment for the enhanced stability, reuseability, biocatalytic activity and biocompatibility have to be taken up.