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"STUDIES ON *STREPTOMYCES MOBARAENSIS* TRANSGLUTAMINASE FOR BIOTECHNOLOGICAL APPLICATIONS"

The thesis encompasses the detailed study of transglutaminase produced by the actinomycetes *Streptomyces mobarensis* NCIM 5208, and its novel applications. Microbial transglutaminases (MTGases) are major cross-linking enzymes which modify the properties of cellular proteins by creating iso-peptide bonds among them. This enzyme has huge potential for applications in the food bioprocessing, pharmaceutical, tissue engineering, and therapeutics. However, these applications necessitate them to be stable at high temperature, and adverse pH environment vis-à-vis their viable production and cost-effective availability, which would otherwise limit wide application in industries. An enzyme of microbial origin is now commonly exploited to overcome the shortcomings of conventionally produced transglutaminases, gaining colossal attention for diverse applications. Therefore, current research efforts have been directed to the bio-process development of MTGase production, regulatory mechanism, nano- immobilization, and their application in tissue scaffold and drug delivery.

MTGase, being nature's biological glue on account of forming ε-(γ-glutamyl) lysine bonds in proteins; however, low productivity and the high cost of production are the major bottlenecks for industrial MTGase production. The present work dealt with these obstacles by enhancing the MTGase production through media engineering using *S. mobaraensis* via a waste valorization approach utilizing agro-wastes. Enhancement of MTGase production (4-fold) vis-à-vis cost reduction was attempted by utilizing a lignocellulosic residue (wheat bran) as the main carbon source for solid state fermentation. In addition, the effect of key regulators in augmenting transcriptional expression of MTGase was elucidated by RT-PCR to unravel the regulatory mechanism of MTGase synthesis in *S. mobaraensis*. The work also intended to explore the nano-immobilization of MTGase for developing stable enzyme bioconjugates. The thesis further deals with various applications of this innovative MTGase preparation for cross-linking food proteins and developing bioinspired tissue scaffolds using casein and gelatin-carbon nanotubes as model systems. The structural and functional changes during/after cross-linking were studied by SDS-PAGE, CD, FS, FTIR, and ATR. The nano-immobilized formulations of MTGase possessed enhanced biochemical and kinetic attributes. Furthermore, cloning, overexpression, and purification were attempted as an alternate approach to overproduce MTGase. The comprehensive study was conducted on the biochemical and structural characterization of purified and recombinant MTGases.

As an innovative part of the thesis, the novel nanoflowers of MTGase could be developed by exploiting the inherent cross-linking activity of this enzyme. Nanoflowers are nano-crystals with floral architecture formed by intra-molecular interactions between the enzymes/proteins. They are endowed with diverse functionalities for biocatalytic applications. The designing of bio-catalytically active enzymatic MTGase nanoflowers (NFs) from the microbial source at a low temperature of 4 ºC has been patented. The study features a new and elegant approach in enzyme immobilization. The novelty in the present work relates to the method of synthesis, the designing of the product, and the process optimization to get the desired features in the NFs. The multifunctional facets of NFs, including simple design and preparation, size tunability, and resistance to enzymatic degradation, allowing customized cargo loading (heavy metals/pollutants/drugs), specific recognition, and internalization into target cancer cells (MCF-7 breast and GBM LN-229 brain) were worked out and formed the major highlights of the thesis.

In a nutshell, the work in the present thesis demonstrated the applicability of MTGase produced by various cost-effective approaches justified the MTGase as an enigmatic enzyme in myriad realms of applications.