SUMMARY

The enormity of hepatic disorder caused by adverse drug reactions has been multifolded dramatically over the past decade. A number of multinational groups like Amphioxus, Invitrogen, Astex, Codexis, Affimetrix, TNO Pharma, are engaged in manufacturing CYP450s from human and animal liver cell culture. The global death rate due to hepatic disorder is about 1.5 billion per year. In developing countries like India, this problem is in higher order as compare to the developed countries. Fortunately, a soil bacterium, *Bacillus megaterium* is having P450BM3 monooxygenase, having 52% homology to human liver origin CYP4503A4. From preliminary trials it has been understood that this enzyme would be immensely helpful as a substitute of human CYP450s, and particularly of CYP3A4. The CYP3A4 content in human liver is about 31% of the total different types of monooxygenases present in liver. So, the global players are keen to develop bacterial origin CYP450s which would be successfully used to prevent adverse drug reactions and other liver metabolic disorder. Keeping in view the above facts and figures the present project has been undertaken as a problem for PhD having target to develop effective microbial process technology to produce CYP450BM3 at scale up level, under low cost budget.

The contents of this thesis give a concise study of manufacturing high value added enzyme, CYP450BM3 using a grass bacterial strain as biological catalyst. Low cost nutritional requirements were used for cultivation of microbe at reactor level. The investigator analyzes both the microbial process technology, and an effective downstream process to obtain purest form of biopharma grade CYP450BM3 both in quality and quantity wise.

Chapter 1 gives an account of brief introduction on structure and function of CYP450. Beside this, the genomic and proteomic relation between CYP3A4 and CYPBM3 were studied to understand the possibility of CYPBM3 as an effective pharmacophore for the control of hepatic disorder and adverse drug reactions. The present market strategy was briefly summarized in the form of table. At the end of chapter, briefly the future prospectives of CYPBM3 as pharmacophore is lucidly described.
Chapter 2 describes the process of obtaining potent mutant(s) from wild strain by physico-chemical mutagen technique. Interestingly, a novel mutant of *B. megaterium* was obtained having high potential for CYP450BM3 biosynthesis by chemical and physical mutagenic treatment, in combination. This newly isolated mutant was proven as a stable strain having high specific rate of P450BM3 enzyme production i.e. 7 folds higher production, as compared to wild variety.

The variation in morphology, ultra structure and biomass composition of wild and mutant strains of *B. megaterium* is critically reviewed in the third chapter. The chemical mutagen resistant bacterial strains were having entirely changed morphology and also color. The variation in color of bacterial colony could be a marker for identifying the nature of selection pressure. Besides, the changes in morphological characters of bacterial cell were also accompanied by alternation in ultra structure of the bacterial cells. The mutagen resistance bacterial cells were having more inclusion bodies that were densely distributed throughout the cytoplasm. The inclusion body carries polyhydroxybutyrate (PHB), a source of generation of redox potential might have responsible for activation of CYP450BM3 enzyme production. The number of vacuoles was noticed to be more in mutagen resistant cells, as compared to the wild strain. In addition, the mutagen resistant cell membrane was noticed to be thinner than the wild variety.

Chapter 4 explains the kinetic of growth and development of mutant bacterial cell as compared to the wild variety. Interestingly, it was noticed that a significant shift in time of appearance of steady state occurred in mutagen resistant bacterial cells. The optimum nutritional and physical conditions required for maximum production of CYP450BM3 were conducted by Plackett–Burman method and subsequently optimized the critical conditions by hybrid design methodology. Eleven variables including two dummy variables were taken in consideration. Three statistically significant nutrients i.e., yeast extract, glycerol and MgSO₄.

The entire downstream process and recovery of enzyme monooxygenase that is CYP450BM3 were given in the chapter 5th and 6th. The cell lysis by high pressure homogenizer was proven to be an economical process as compared to cell disruption by sonication process in alkaline environment. The separation of sonicated bacterial cells was carried by 300 mesh filter cloth coated with activated charcoal. The crude enzyme extract thereafter was precipitated with 35% ammonium sulphate at 4°C. The
process of purification was carried out at 4°C by column chromatography by using DEAE sepharose. The crystallized enzyme in its purest form was obtained thereafter was subjected to low angle X-ray crystallography to understand the folding nature of the enzyme. The concluding chapter explains the feasibility of manufacturing this enzyme at commercial level.

In the investigator opinion this information should provide immense benefit to the biotechnology based industries engaged in manufacturing CYP450s at commercial level.

**FUTURE STRATEGY:**

1. Characterization of folding nature of CYPBM3 by low angle X-Ray crystallography followed by computational processing to understand the substrate binding sites of CYPBM3.

2. Immobilization of CYPBM3 on bacterial membrane fragments.

3. In vitro, process for 3-DMC and 3-DMTC production.

4. In vitro, process for glycosylation of 3-DMC and 3-DMTC.

5. CYPBM3 embroidered impeller designing.