PREFACE

L-Glutaminase (glutamine amidohydrolase EC 3.5.1.2) catalyzes the hydrolysis of L-glutamine into glutamic acid and ammonia. In pharmaceutical industy, this enzyme is used as an excellent antitumor agent by depriving glutamine-dependent tumor cells with the essential nutrient, glutamine (Pal and Maity, 1992). It is also used for treatment of acute lymphocytic leukemia and HIV (Roberts et al., 2001). In food industry, it is used as flavor enhancer for producing sharp taste in fermented Chinese foods such as soy sauce (Sabu et al., 2000, Iyer and Singhal, 2008) and production of L-theanine in Japanese green tea which has antihypertensive property and also improves the effects of antitumor agents by suppressing stimulation by caffeine (Tachiki et al., 1996). In recent years, L-glutaminase based biosensors are used for monitoring the glutamine level in mammalian and hybridoma cell cultures (Sabu et al., 2000). Although L-glutaminase is produced by almost all living cells for catabolism of glutamine, microbial L-glutaminase has received greater attention for its potential biotechnological applications and easiness in large scale production.

In the present thesis report, the production of L-glutaminase from a new high yielding bacterial strain of *Bacillus cereus* MTCC 1305 and its anticancer property was studied. The general introduction and literature review of study of L-glutaminase is reported in **Chapter 1** and **Chapter 2** respectively.

Chapter 3 deals the selection of high yielding bacterial strain for producing extracellular L-glutaminase. In this chapter, the reaction conditions were also optimized using statistical tools like response surface methodology (RSM) and artificial neural network (ANN).

Chapter 4 deals the optimization of culture conditions and media components for production of L-glutaminase from *Bacillus cereus* MTCC 1305. Effect of different amino acids, carbon and nitrogen sources on its production were further studied. The statistical tools, RSM and ANN, were employed to optimize media components and cultural conditions to achieve maximum production of L-glutaminase

Chapter 5 deals the investigation of effects of aeration and agitation rates on the production of L-glutaminase production from *Bacillus cerues* MTCC 1305 in bioreactor. The oxygen transfer characteristic parameters of the fermentation process were further determined at optimum level of agitation and aeration rate. Kinetic models were developed for L-glutaminase fermentation using Logistic equation, Luedeking-Piret equation and Modified Luedeking-Piret equation for cell growth, product formation and substrate consumption respectively..

Chapter 6, deals the study of extractive fermentative production of L-glutaminase from *Bacillus cerues* MTCC 1305 using different PEG/salts or PEG/dextran system. The effect of fermentation time, pH and temperature on the extractive production of L-glutaminase was also studied.

Chapter 7 deals the purification and biochemical characterization of L-glutaminase produced from *Bacillus cereus* MTCC 1305. The enzyme was purified to homogeneity using different purification steps like ammonium sulphate precipitation, dialysis membrane, DEAE cellulose chromatography, SDS-PAGE and native-PAGE. The effect of pH, temperature, different metal ions, EDTA, thiol binding agents and reducing agents on the activity of the purified enzyme was studied. The thermostability, thermal deactivation and substrate specificity of the purified enzyme was also studied.

Chapter 8 deals the homology modeling of the purified L-glutaminase and its docking analysis with L-glutamine substrate. MALDI-TOF/TOF analyzer was first used to obtain the peptide sequences of L-glutaminase from *Bacillus cereus* MTCC 1305. BLAST search of these peptide sequences against Protein Data Bank was performed to search the possible template model, which was further used to predict 3D structure for L-glutaminase from *Bacillus cereus* MTCC 1305. ANOLEA, QMEAN4 global scores, Gromos 96 and PROCHEK tools were further applied to validate the predicted model structure. Docking analysis of the predicted model structure with L-glutamine as substrate was further studied using SWISS dock tool (http://swissdock.vital-it.ch/).

Chapter 9 deals the study of effect of different concentration of purified L-glutaminase on the growth of human cancer cell lines such as, hepatocellular carcinoma (Hep-G2), colon carcinoma (HCT-116).