Summary and Conclusion
6. SUMMARY AND CONCLUSION

Among the major human diseases, cancer is a most dangerous disease; cancer the very name alone scares most people. The cancers are smart and cagey buggers. They can hide and grow for a good spell undetected. It is the second biggest disease of the human beings. The number of deaths due to cancer in developed countries is three time’s greater than the number (85 per 100,000 populations) in developing countries. As per World Cancer Report, cancer rates could further increase by 50% to 15 million in 2020.

Many enzymes have been used as drugs like wise L-asparaginase attracted much attention because of its use as effective therapeutic agent against lymphocytic leukemia and other kinds of cancer in man L-asparaginase breaks down (disassembles) the amino acid asparagine which is needed for cell maintenance and growth. In many cases of leukemia cells are unable to make their own asparagine and must rely on outside source of asparagine for survival. By depleting free asparagine in the body, which is necessary for cancer cell, results in a depletion of cancerous cells while normal cells are more likely to be preserved. Therefore, the discovery of new L-asparaginase is utmost important. Traditionally, the L-asparaginase is being produced by means of submerged fermentation, but there are no reports on the production of L-asparaginase through solid state fermentation (SSF) by employing molds. Therefore, in the present study attempts have been made on the suitability and utility of carob pods as substrate for the production of L-asparaginase by employing locally isolated strain of Aspergillus terreus.
The summary of the results obtained and the conclusions arrived at on the basis of these results presented in the present thesis are briefly outlined as below:

1. The Chapter I includes a brief introduction to the research undertaken, wherein the necessity of undertaking the work is justified and the aims and objectives of the study are specified.

2. In Chapter II, an exhaustive review of the relevant aspects of L-asparaginase chemistry and historical aspects, the characteristics of the L-asparaginase and substrates, etc., are presented and also an attempt has been made to evaluate the different substrates used in fermentation for production of L-asparaginase.

3. The Chapter III deals with the materials used and methodology adopted in the present study. The aspects covered are mainly – isolation, screening, the chemical analysis of agro-based waste carob pod, solid state fermentation procedure and methodology adopted to optimize the fermentation parameters, as well as process economization through nutrient supplementation and mutation of *A. terreus* KLS2 for maximum production of L-asparaginase.

4. Purification and characterization of L-asparaginase

5. In the Chapter IV, the results obtained during the study are presented. The results are indicated in brief as below:

i. The isolates of *A. terreus* were initially subjected for L-asparaginase production through rapid plate assay by evaluating their zone of clearance. All thirty five 35 isolates, exhibited zone of diameter. Of these one isolate *A. terreus* KLS2 exhibited zone of diameter of
0.98 cm and hence the same was considered as the promising strain for L-asparaginase production.

ii. Screening of various agricultural substrates, like rice chaff (husk), pigeon pea waste, groundnut cake, banana wastes as well as carob (Ceratonia siliqua) pod, were carried out. The results of suitability of substrate for solid state fermentation indicated that carob pod was suitable substrate for L-asparaginase production.

iii. The success and direction of fermentation depends on obtaining a proper balance between the substrate, the process itself and the fermenting organisms. Hence, optimization of solid state fermentation parameters like initial moisture content, pH of the substrate, the ambient temperature and the inoculum size, particle size of the substrate, bed depth on L-asparaginase production was carried out. Once a parameter was optimized, the optimum level of the parameter was continued in the next step of experiment.

a. The studies reveal that the optimum fermentation period required for production of maximum (6.05 IU) L-asparaginase form carob substrate was 72 hrs by employing A. terreus KLS2.

b. The optimum initial moisture content for maximum production of L-asparaginase by the organism was observed at 65%, like wise pH of 4.5, temperature of 35°C, inoculum size of 1 x 10⁷ spores/ml, particle size of 2 mm and bed depth of 30 mm were
observed as optimum for maximum production of L-asparaginase using deseeded carob pods as substrate.

iv. In the studies dealing with the process economization, attempts have been made to improve the production of L-asparaginase during solid state fermentation of the carob pod substrate through supplementation of various nutrients and also by mutation of the fermenting organism.

In the studies involving nutrient supplementations, the substrate was amended with varying concentrations of different carbon sources, organic and inorganic nitrogen, phosphates, lower alcohols, metal ions individually and also in step-wise addition of each of these nutrients in their optimum levels.

a. Amongst the carbon sources tested for L-asparaginase production glucose proved to be beneficial. The optimum concentration of glucose needed to be supplemented to the carob pod substrate to effect maximum L-asparaginase production by *A. terreus* KLS2 was observed to be 0.15 M. At this level of glucose supplementation, *A. terreus* KLS2 produced 7.45 IU of L-asparaginase at 72 hrs of fermentation.

b. The optimum concentration of inorganic nitrogen needed to be supplemented to the carob pod substrate to cause maximum L-asparaginase production by *A. terreus* KLS2 was observed to be 0.13%. At this level of ammonium sulphate supplementation, *A. terreus* KLS2 produced 6.64 IU 72 hrs of fermentation.
c. Amongst the organic nitrogen sources tested for L-asparaginase production yeast extract proved to be beneficial. The optimum concentration of yeast extract needed to be supplemented to the carob pod substrate to effect maximum production of L-asparaginase by *A. terreus* KLS2 was observed to be 1.0%. At this level of yeast extract supplementation, *A. terreus* KLS2 produced 6.117IU at 72 hrs of fermentation.

d. Amongst all the phosphate sources tested for L-asparaginase production, diammonium hydrogen phosphate proved to be beneficial. The optimum concentration of diammonium hydrogen phosphate needed to be supplemented to the carob pod substrate to effect maximum production of L-asparaginase by *A. terreus* KLS2 was observed to be 0.10M. At this level of diammonium hydrogen phosphate supplementation, *A. terreus* KLS2 produced 6.20 IU at 72 hrs of fermentation.

e. Amongst the alcohol sources tested for L-asparaginase production glycerol proved to be beneficial. The optimum concentration of glycerol needed to be supplemented to the carob pod substrate to effect maximum L-production production by *A. terreus* KLS2 was observed to be 0.50%. At this level of glycerol supplementation, *A. terreus* KLS2 produced 7.11 IU at 72 hrs of fermentation.
f. Amongst all the metal ions tested for maximum L-asparaginase production manganous sulphate proved to be beneficial. The optimum concentration of manganous sulphate needed to be supplemented to the carob pod substrate to effect maximum L-asparaginase production by *A. terreus* KLS2 was observed to be 0.1%. At this level of manganous sulphate supplementation, *A. terreus* KLS2 produced 6.66 IU at 72 hrs of fermentation.

g. In the studies involving step-wise addition of each nutrient (at optimum levels as observed previously) it was observed that a step-wise increase in the L-asparaginase production from the carob pod substrate by *A. terreus* KLS2. Upon supplementation of optimum levels of the nutrients maximum production of L-asparaginase (7.95IU) was effected by *A. terreus* KLS2. The production of L-asparaginase by the organism increased as one nutrient after another nutrient was added in the stepwise order VI >V > IV > III > II > I.

h. Induction of mutation was attempted by UV rays treatment at distance of 10 cm for 10 min. The mutant strain exhibited zone of clearance of 0.98 cm on rapid plate assay. Based on the performance of the strain obtained on rapid plate assay, the strain *A. terreus* KLS 2 mu was selected for further studies.
i. The results obtained after solid state fermentation revealed that the mutant strains yielded 8.15IU of L-asparaginase when compared to the parent strain *A. terreus* KLS2, which has yielded 6.05IU on deseeded carob substrate for 72 hrs fermentation.

j. The purification studies indicated that the crude enzyme extract showed highest specific enzyme activity of 2.6 U/mg proteins, whereas ammonium sulphate precipitation, DEAE- cellulose (Ion-exchange) and Sephacryl S-200 (gel filtration) chromatography showed highest specific enzyme activity of 3.4 U/mg proteins, 10.4 U/mg proteins and 11.5 U/mg proteins respectively. In the ammonium sulphate precipitation method about 1.27 fold increases in the specific enzyme activity was observed with 20.24% recovery of proteins. Similarly, in DEAE-cellulose (Ion-exchange) and Sephacryl S-200 gel filtration chromatography about 4.0 and 4.42 f olds increase in the specific enzyme activity with 4.29% and 3.80% recovery of proteins was observed respectively.

k. The enzyme characteristics studies indicated that the enzyme is optimum at alkaline pH of 9.0 and the optimum temperature was found to be 37°C. The studies also indicated that it is stable at alkaline pH of 8.0 at 70°C and it is quite specific for its natural substrate i.e., asparagine.
5. In Chapter V, a detailed discussion of the findings of the present work is made with reference to the works done by the earlier workers in this field. The present study indicated that the substrate, carob pod, is suitable for the production of L-asparaginase by employing *A. terreus* KLS2 strain under solid state fermentation.

6. The bibliography referred in the present thesis is included in the References section at the end of the thesis.