CHAPTER-VII

SUMMARY AND CONCLUSION
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Actinomycetes are a large group of Gram positive eubacteria present in a wide range of terrestrial and marine environments. They are prolific producers of antibiotics and other secondary metabolites. Actinomycetes are reported to produce a number of enzymes among which proteases occupy a significant position.

In the present study an attempt has been made to isolate protease producing actinomycetes from different soil and water samples. A promising isolate GAS-4 was obtained from a soil sample collected from Sangam dairy, Jagarlamudi in Guntur district and it initially produced 80U/ml of protease. Initially protease was produced in submerged fermentation in media reported in literature and later in modified production media when the yield of protease increased to 92.3U/ml. The yield of protease was increased to 119.4U/ml and 132.5U/ml after mutation by UV and HNO\textsubscript{2} respectively.

The yield of protease was improved by employing statistical methods like PBD. For this 9 variables were selected and an experimental plan with twelve runs was designed. A yield of 144.8U/ml was obtained with the following conditions: pH 7, Inoculum 5%, temperature 28°C, rpm 180, Age of inoculum 36 h, Incubation period 96 h, Glucose 1%, Yeast extract 1% and tryptone 1%. The analysis of the data revealed that only temperature is contributing very significantly to protease production. pH and Incubation period are the other variables whose contribution is 2.36 and 1.68 respectively.

Subsequently a plan was designed for these three variables viz. Temperature, pH and incubation period with high level, midpoint and low levels. Maximum yield of protease 153.2 U/ml was observed in the eighth run with X1, X2, X3 being at
temperature 28°C, pH 9.0 and 96 h incubation period. The yield of protease increased from 80 Units (Parent strain) to 153.2 U/ml which represents an increase of 87.5%. Isolate GAS-4 was identified as *S. indicus* after evaluation of biochemical properties and 16S rRNA sequencing. We observed some differences in biochemical properties between the reference strain 1H32-1(T) reported by Luo *et al* and our isolate. The differences were observed in utilization of Xylose, Nitrate reduction and Mannitol. Hence we designated our isolate as *S. indicus* var. GAS-4.

The enzyme was purified from 2 ltr fermentation broth by Ammonium sulphate precipitation, dialysis, ion exchange chromatography and gel filtration. The molecular weight of the enzyme determined by Native PAGE was 60KDa. The enzyme activity was assessed by Zymography method. The 3D structure of the enzyme was determined by ROSOMAL method. The enzyme had an optimum pH 9.0 and it was completely inhibited by PMSF (Phenyl Methyl Sulphonyl Fluoride) indicating that it was a serine alkaline protease. The $K_m$ of the enzyme was 6.4mg which is equivalent to $1.06 \times 10^{-4}$M and the $V_{max}$ was 0.54IU.

The purified enzyme was used in dehairing goat skin and considerable loosening of hair was observed by applying moderate force. Washing performance of the alkaline protease in the presence of detergent was assessed by the removal of blood from a stained cloth. In the presence of the enzyme and detergent the stain was almost completely removed. The compatibility of the enzyme with different detergents was evaluated and it was found that Aerial was the best detergent. The purified enzyme exhibited enhanced activity in the presence of Ca++ and Na+ ions. This is advantageous when the enzyme is used industrially. In this study a serine alkaline protease was produced by a variant of *Streptomyces indicus* GAS-4.

The yield of enzyme after optimization of nutritional and cultural conditions increased from...
80U/ml (parent strain) to 153.2 U/ml which represents an increasing 87.5%. The yield of alkaline protease is better or comparable to those reported in the literature. The results obtained in this study indicate the scope for utilization of this enzyme in industry after pilot plant and scale-up studies are conducted.