PREFACE

Processed leather constitute major export commodity from India (about 300 million tons skins made every year), the leather industry makes great profits while also bringing significant environmental pollution. Therefore, it is urgent that clean and eco-friendly technologies be developed for leather processing and effluent treatment. Use of enzymes in place of hazardous chemicals at different stages of leather processing, especially in dehairing, which brings almost 80%–90% of the pollution in the whole processing, is being developed.

Compared with traditional chemical methods, enzymatic processes not only yield quality-improved products, but also reduce the use of hazardous and polluting chemicals. However, many proteases especially conventional are not suitable for dehairing, since they often lose their activity in higher temperature in the course of process. Therefore, it is highly essential to find proteases having stable activity while maintaining high dehairing activity.

Leather industry contributes to one of the major industrial pollution problems faced by the country, and the pollution causing chemicals, viz. lime, sodium sulphide, salt, solvents, etc. arise mainly from the pre-tanning processes of leather processing. In order to overcome the hazards caused by the tannery effluents, use of enzymes as a viable alternative has been resorted to in pre-tanning operations such as soaking, dehairing, bating,
degreasing and offal treatment. Besides being expensive and particularly unpleasant to carry out, a strongly polluting effluent is produced. The alternative to this process is enzyme-assisted dehairing. Enzyme-assisted dehairing is preferentially possible if proteolytic enzymes can be found that are stable and active under the alkaline conditions of tanning.

Proteases may play a vital role in these treatments by replacing these hazardous chemicals especially involved in soaking, dehairing and bating. Increased usage of enzymes for dehairing and bating not only prevents pollution problems, but also is effective in saving energy. In general, proteases play a vital role in leather processing starting from soaking of hides to finished products. With these enormous benefits many of protease work for very short time period as in the course of process often they will lose their activity due to long run of reaction, present of inhibitors, alteration of pH and mainly due to higher temperature. To tackle these problems thermostable proteases have been used significantly large scale in last one decade. The use of thermostable protease as leather dehairing enzymes for unhairing skin and hides significantly minimize stability problem of conventional protease without any negative impact on leather production, leather quality environment.

Though the classes of thermostable proteases have shown tremendous benefits for industrial applications and especially for leather processing but the production of these proteases is still a major drawback.
As the protease is a group of the different enzyme like serine, alkaline and acid protease. As their habitat it’s quite difficult to grow in higher temperature for large scale production and subsequent purification. Hence, this study is working for molecular cloning and expression analysis of these thermostable proteases in the *E. coli* system which is quite convenient for large scale production. This study helps in looking for the over expression of these proteases in the host system specially designed for recombinant protein to fulfill industrial demand.

The engineered strains come up with great potential of producing recombinant protein in desired quantity. The major drawback in conventional expression system like *E. coli* BL21 (DE3) is protein toxicity led death of expression host system in higher level of protein expression. Genetically designed stains like *E. coli* C41 (DE3), C43 (DE3) and Rosetta come up with enormous potential for commercial production of recombinant protein. The refined strains offer not only over expression of desired recombinant protein but also provides ideal platform for expression of both prokaryotic and eukaryotic sourced protein. Newer generations of expression host system such as *E. coli* 41 (DE3), C43 (DE3) and Rosetta possess extra universal codon essentially needed for expression of diverse sourced protein.

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