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Biotechnology Research Center
مركز التكنولوجيا الحيوية للأبحاث

Cloning and expression of a serine alkaline protease for leather industry

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Background

The industrial sector plays a significant role in the process of economic development in Palestine. Leather industry was considered as one of the largest industrial sectors until the end of last decade. In Palestine, there are 18 tanneries, 10 of them are in Hebron city and some in Nablus[1-2].

Although tannery is considered one of the important industrial sectors, it is the main environmental polluter (Fig. 1). Conventional dehairing processes of leather industry release vast amount of pollutants that contribute 80-90% of the total pollution in the industry which generate noxious gases such as hydrogen sulfide and solid wastes such as lime and chrome sludge [3] (Fig. 2). This type of pollution is considered the major contributor to water pollution for this reason, the tanneries in Hebron are responsible for remarkable environmental impacts. However, pollution from tanneries, similar to other major industries, pose long-term negative environmental impacts irrespective of the immediate economic benefits they generate [1].

In order to overcome this problem; the use of bio-catalysts, such as engineered recombinant enzymes, in replacement of harmful industrial synthetic chemical products is the solution. Of the many industrial enzymes, Proteases represent one of the three largest groups and account for about 60% of the total worldwide sale of enzymes [4]. Proteases have a long history of application in the food and detergent industries. Their application in the leather industry for dehairing and bating of hides to substitute currently used toxic chemicals is a relatively new development and has conferred added biotechnological importance [5]. Most commercial proteases, mainly alkaline, are produced by organisms belonging to the genus *Bacillus* and the best known is serine alkaline protease produced by *Bacillus licheniformis* [4, 6].

Here we describe our progress to-date in the production of an alkaline protease from *Bacillus licheniformis*.

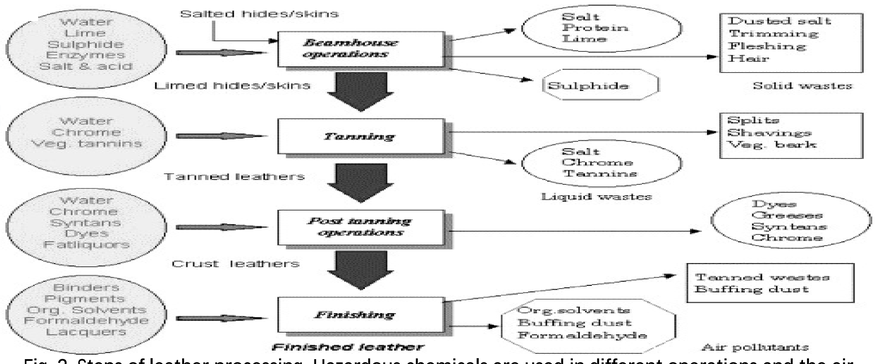


Fig. 2. Steps of leather processing. Hazardous chemicals are used in different operations and the air pollutants generated in each operation.

Results and discussion

A *Bacillus licheniformis* bacterial strain has been successfully isolated from soil of poultry. Chicken feather is an alkaline environment, thus bacteria that are commonly living in this environment are thought to be alkaline as well. *Bacillus* containing an alkaline protease was tested using skim milk agar media and a large clear areas were shown (Fig. 3), an indication of protease activity. Following *Bacillus* genomic DNA extraction, a 1.14 Kb serine alkaline protease gene was amplified (Fig. 4) and cloned into cloning vector, pGEM-T-Easy. The cloning vector with the protease gene cloned to it was then transformed into *DH5α E. Coli* strain and plated on agar plates containing ampicillin and x-gal. A positive clone was identified on the selection medium (Fig. 5A). The white colony was cultured and PCR was conducted on intact cells to screen for colonies with protease gene (Fig. 5B). The size was similar to the expected 1.14 kb protease gene. Positive clones were cultured and glycerol stocks prepared. Plasmid was then isolated and sent for sequencing. The protease gene will be transferred into an expression vector to produce the enzyme and test its activity. The work is under way to express the protein and test its quality. This project is expected to help the leather industry in Palestine and help reduce the pollution and hazardes resulted from the exposure of workers to toxic chemicals in conventional tanneries (Fig. 6).

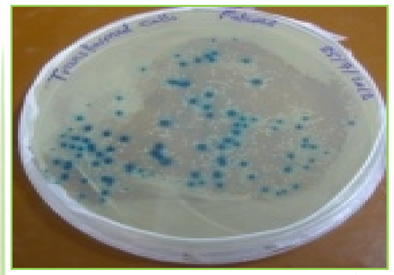


Fig. 5A. Verification of positive cloning in pGEM®T-Easy cloning vector by blue-white selection

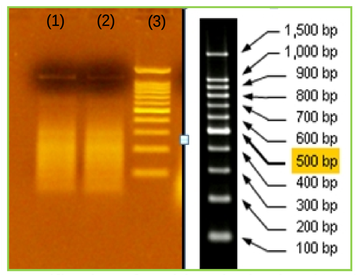


Fig. 5B. Verification of positive cloning in pGEM®T-Easy cloning vector using PCR. The gene matches the correct protease size of 1.14 kb. Bands1 and 2 are the protease gene from different clines. Band #3 is the molecular ladder.

Conclusion

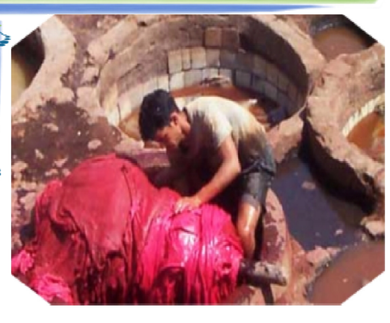
An alkaline protease was cloned from *Bacillus licheniformis*. The use of serine alkaline protease in leather industry will replace the harmful conventional chemicals that have been used for tanning and dehairing operations. Alkaline protease will speed up the process of dehairing because the alkaline conditions enable the swelling of hair root and the subsequent attack of protease on hair follicle proteins allows easy removal of the hair.

Fig.6. Worker at a tannery showing leather during tanning process

AKNOLEDGMENT

I would like to thank the staff of the Biotechnology Research Center for their help and support.

F.g.1. Conventional leather industry is considered one of the major pollutants to environment. The excessive use of toxic chemicals expose works to hazards as well.



Project goal and objectives:

The overall objective of this study is to provide an environmentally friendly product for local industries in Palestine.

Specific objectives:

To clone and express a *Bacillus licheniformis* alkaline serine protease.

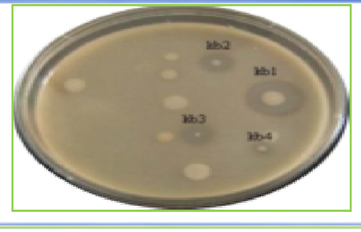


Fig. 3. The zones of clearance on skim milk agar plate produced by *Bacillus licheniformis* after incubation at 30 ċ for 48 hours .

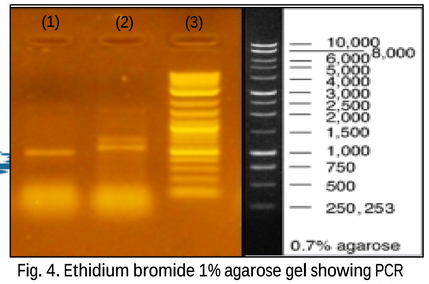


Fig. 4. Ethidium bromide 1% agarose gel showing PCR products match the size of the protease gene of 1141bp. Lane 1-2 :PCR amplified protease gene. Lane 3 is the molecular ladder.

Methodology

This work has been carried out in two main stages: first, the isolation of *Bacillus licheniformis* from soil and second cloning and expression of serine alkaline protease gene from *Bacillus*.

Strain isolation

Samples of soil were taken from a poultry farm. Serial dilution of the sample was prepared by adding 1g of the soil to 9 ml of sterile saline. The last dilution was plated on nutrient agar medium and incubated at 28 ċ for 24 hours. The colonies appeared were plated on skim milk selection agar media, then incubated at 30 ċ for 48 hours. The colonies that resulted in high areas of clearance were selected and sub-cultured in nutrient agar broth.

Cloning and expression of gene encoding serine alkaline protease.

Serine alkaline protease gene was PCR-amplified using conserved primers and cloned into pGEM®-T-Easy cloning vector. Following verification by sequencing the DNA will be sub-cloned into pPROEXHTb expression vector. Recombinant protease will be tested for activity and a quality control test will be carried out.

References:

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