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Isolation of Protease Producing Bacteria from Soil and Genomic Library Construction of Protease Gene

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Abstract: Six soil samples were collected from various areas of Chandigarh and Kharar, out of which 3 were waste soil samples and 3 garden soil samples. In all, 16 isolates from the garden soil and the waste soils were processed for the isolation of bacterial species. *Bacillus* was selected as the major producer of proteases. The optimum temperature and pH for the growth of this genera was found to be 45ºC and 7 respectively by Well Assay Method. Gram staining and microbial study of microorganisms was done to determine the species. The screened microbial colonies revealed the protease producing species of *Bacillus.* A genomic DNA library was constructed and screened to obtain the corresponding protease gene. It is revealed from the present study that this protease shows high activity on skimmed milk plates by generating a clear zone of hydrolysis.

Keywords: *Bacillus,* Genomic library, Protease gene, Isolation.

INTRODUCTION

Proteases are hydrolytic enzymes that hydrolyze proteins by adding water across peptide bonds and break them in smaller peptides in organic solvents[1]. The proteases play a critical role in many physiological and pathological processes such as protein catabolism, blood coagulation, cell growth and migration, tissue arrangement, morphogenesis in development, inflammation, and tumor growth, activation of zymogens and transport of secretary proteins across the membranes[2]. Around 30% to 40% of the cost of enzyme depends upon the cost of the medium[3]. The enzymatic yield obtained

from fermentation, cost of their production and downstream processing cost determines the final cost of the enzyme produced[4]. For industrial usage proteases can be obtained by fermentation from animal tissues, plant cells and microbial cells[5].

The major objectives of the present study were isolation of protease producing bacteria, optimization of bacteria and gene cloning. Proteases are one of the standard ingredients of all kinds of detergents ranging from those used for household laundering to reagents used for cleaning contact lenses or dentures. The use of enzymes has also proved successful in improving leather quality, in reducing environmental pollution[6],for cheese making, baking, preparation of soya hydrolyses, meat tenderization and the oral administration of proteases as a digestive aid to correct certain lytic enzyme deficiency syndromes[7].

MATERIALS AND METHODS

Soil samples were collected in small polythene bags from different areas of Chandigarh and Kharar, for the isolation of protease producing bacteria. The soil samples were taken to Laboratory of Biopharma Department, Chandigarh Group of Colleges, Gharuan (Mohali).

Media Preparation, isolation, screening and streaking of protease producing microorganism: The sample collected from different location was subjected to serial dilutions for the isolation of micro-organism. Three dilutions i.e.10^{-3} , 10^{-5} and 10^{-7} were selected for further analysis .Screening and streaking of protease producing microorganism was done on nutrient agar plates containing skim milk as substrate. The gram staining was done using different dyes. Then optimization of temperature and pH was done.

DNA Isolation: Genomic DNA was isolated using Tris-HCl, EDTA, Glucose, Proteinase K, SDS, autoclaved water, Phenol, Chloroform, Isoamylalcohol, TE Buffer in various concentrations using standard procedure. Then agarose gel electrophoresis was done. Spectrophometric analysis was done to visualize DNA bands. Competent cell preparation was done by $CaCl_2$ method. Transformation was done using pUC19 as vector. Further plasmid DNA was isolated using Tris-HCl, EDTA, Glucose, potassium acetate, NaOH, SDS, Glacialacetic acid, phenol, Chloroform, Isoamylalcoholusing standard procedure, agarose gel electrophoresis and spectrophometric analysis .

Digestion, Ligation and Transformation: A restriction digestion was done using Eco R1, Eco R1 buffer, DNA template, a procedure used in molecular biology to prepare DNA for analysis, genomic library construction (**Fig.4**). The restriction enzyme Eco R1 was used to cleave DNA molecules. Then ligation was done using T4DNA ligase, T4 DNA ligase buffer, restricted DNA, restricted pUC 19 and sterilized water.The ligated samples were used to transform *E.coli* 5 α competent cells by using heat shock method (**Fig .5**).Further,the positive colonies were picked up and streaked on LB ampicillin plates and preserved as a reference for genomic library.Thus a genomic library was constructed and screened to obtain the corresponding protease gene.

RESULTS AND DISCUSSION

The colonial growth was observed on the nutrient agar media after incubating it at 37°C for 24 hrs. Screening of protease producing microorganisms done using skim milk as substrate revealed the clear zones around the colonies (**Fig.1**). This indicated that the strain had the potential to hydrolyze the skim milk present in the medium. Further, from the staining and morphological characters it was observed that the bacterium was *Bacillus sp.* as mostly observed stains were purple or blue in color, rod shaped and gram positive. Protease producing bacteria showed maximum growth at around pH 7 (**Fig.2**) and maximum zone of hydrolysis at temperature 45°C (**Fig.3**).

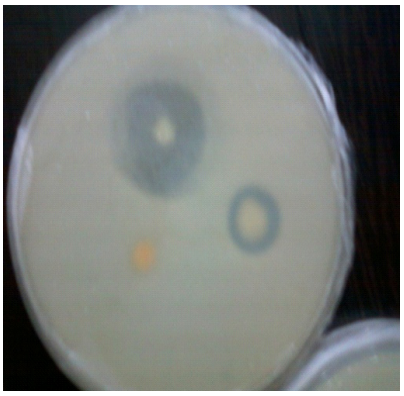

Fig. 1: Zone of hydrolysis produced by protease producing microorganism

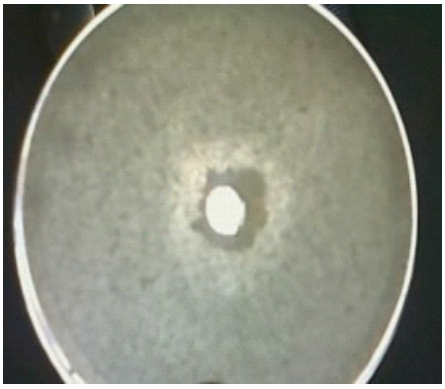

Fig. 2: Hydrolysis zone at pH 7

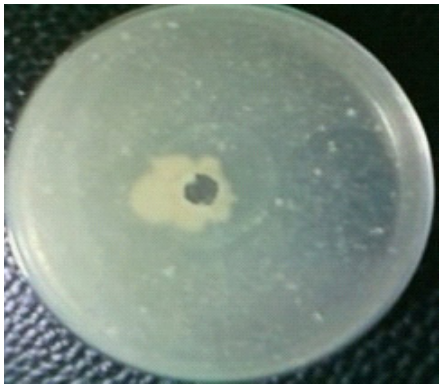

Fig. 3: Hydrolysis at temperature 45°C

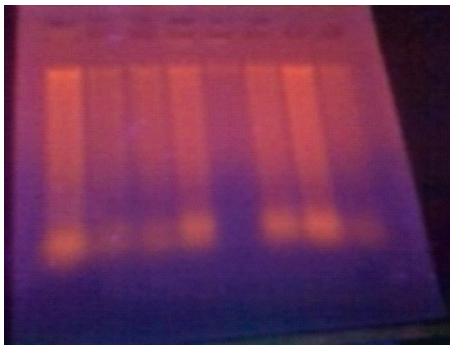

Fig.4: Gel image showing plasmid DNA bands

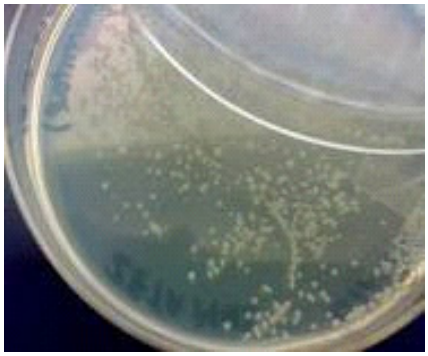

Fig. 5: Petriplate showing transformed colonies

Ligated product was used to transform the cloning host (*E.coli DH5α* competent cells). Further, the positive colonies picked up and streaked on LB ampicillin plates were preserved as a reference for genomic library. Thus a genomic library was constructed and screened to obtain the corresponding protease gene.

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