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Production and Partial Purification of Protease by Actinomyces Species

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Abstract- Enzymes are biocatalysts obtained from plants, animals and microorganism. Microbial enzymes are becoming important for its technical and economical advantages .Various kinds of microorganisms in nature degrade different type of proteins indicating the Proteolytic capabilities of microorganism.

About 80% of enzymes produced annually are simple hydrolytic enzymes, of which 60% are proteases. Different bacteria, fungi and actinomycetes are the major sources of microbial proteases. Extracellular proteases have high commercial value and multiple application in detergent, food, diary, pharmaceutical, leather, diagnostic, waste management and silver recovery industries.

Here we consider the extra cellular protease which is secreted by Actinomycetes Sp. This enzyme is free from most of the toxic agents. And it is considered as a safe/suitable protease for the oral medication purposes. Here we find the best suit Actinomycetes strain which yield high protease, and its suitable Ph and the medium (Plate assay method). Then we find the high Protease production in different SSF and LSF. Thereafter we discuss the protease isolation methods, conformation and their application in the medical and industrial fields.

Index Terms- Actinomycetes, Extra Cellular enzyme, Hydrolytic Enzyme, Protease production

I. INTRODUCTION

Protease constitutes one of the major important groups of industrial enzymes; about 80% of enzymes produced annually are simple hydrolytic enzymes, in which 60% are proteases. Different bacteria, fungi and actinomycetes are the major source of microbial proteases. Extracellular proteases have high commercial value and multiple application in detergent, food, diary, pharmaceutical, leather, diagnostic, waste management and silver recovery. The microbial protease represents 60% of the worldwide market of industrial enzyme. They find commercial application for toothpastes as antiplaque and antitartar, cosmetics and for the recovery of silver from used X-ray films (Ishikawa *et al*.,1993).

The wide diversity and specificity of protease is used to develop effective therapeutic reagents. Proteases are used to treat burns and wounds. Oral administration of proteases produces an anti-inflammatory response in burned patients and speeds up the healing process. Trypsin and chymotrypsin acts as anti-inflammatory and antioxidant agents on burn wounds. (Bitange et al., 2008) Protease deficiency leads to arthritis, osteoporosis and other calcium deficient diseases. Because protein digestion occur and digested protein converted into glucose. This leads to hypoglycemia; resulting in moodiness, mood swings and irritability.

The microbial enzyme (Protease) secretes in the phase of their growth. For the commercial requirement they may obtained as a by-product of antibiotic production.

II. METHODOLOGY

The marine filamentous bacterium *Actinomycetes* was originally isolated from marine sources.

The actinomycetes were streaked on 0.3% casein, gelatin and skim milk. The plates were incubated at room temperature (28°C) for four days. Then the plates were flooded with 15% mercuric chloride solution and 20%HCl for 5min. Then the plates were observed for the development of clear zone. Then the Actinomycetes were grown in broth culture media. The culture was inoculated with 3% of seed culture and incubated in a rotary shaker with the speed of 200rpm. The medium was harvested and collected after 7 days and the amount of protease was estimated. Actinomycetes from culture medium was poured and streaked aseptically in substrate amended agar plates and incubated in room temperature for 10 days. The enzyme activity was visualized as a clear zone of substrate utilization after flooding the plates.

The culture was harvested after 6days. The harvested culture was filtered through whatmann No.1 filter paper. Then the free cells were separated by centrifugation. (10,000rpm for 15 minutes) Supernatant was fractionated by precipitation with ammonium sulfate 80% of saturation. Another harvested culture was suspended by centrifugation, and the supernatant was collected and measured. Fractionated enzyme was precipitated by adding half the amount of ice cold acetone.

The collected protein was dialyzed for two days with 0.1m phosphate buffer. The dialyzed fraction was collected and stored. Protein pellet was dissolved in 0.1M phosphate buffer, (P^H7) and dialyzed. The protein content of the culture was estimated by binding method of Bradford (1976).

The molecular mass of purified protease was determined by SDS-PAGE. Purified protein samples were loaded on SDS-PAGE with the standard protein ladder. (Consists 100 kDa) After the separation, the gels were stained with silver nitrate.

III. RESULTS

A clear zone was observed and the protease Production (by *streptomyces* sp.) was high in casein. The maximum zone was observed on the 6^{th} day.

After 3days a clear zone was measured in the quantitative medium. It shows that the Actinomycetes produce high amount of protease on third day.

The 6th day culture was collected, filtered, and the filtrate was precipitated by 80% ammonium sulfate saturation or acetone. Proteases were harvested by centrifugation at 5000rpm for 25mins.Then the protease was dialyzed for 2days against phosphate buffer and the crude protease was obtained.

Partially purified proteases were separated by using 10%acrylamide in SDS-PAGE. Protease bands were visualized, when the gel was stained with silver nitrate solution. The partially purified protease produces a band on polyacrylamide gel.

Proteases were separated on 10% acrylamide under non-denaturing conditions. Bands were visualized on native gel when stained with Coomosive Brilliant Blue and silver nitrate.

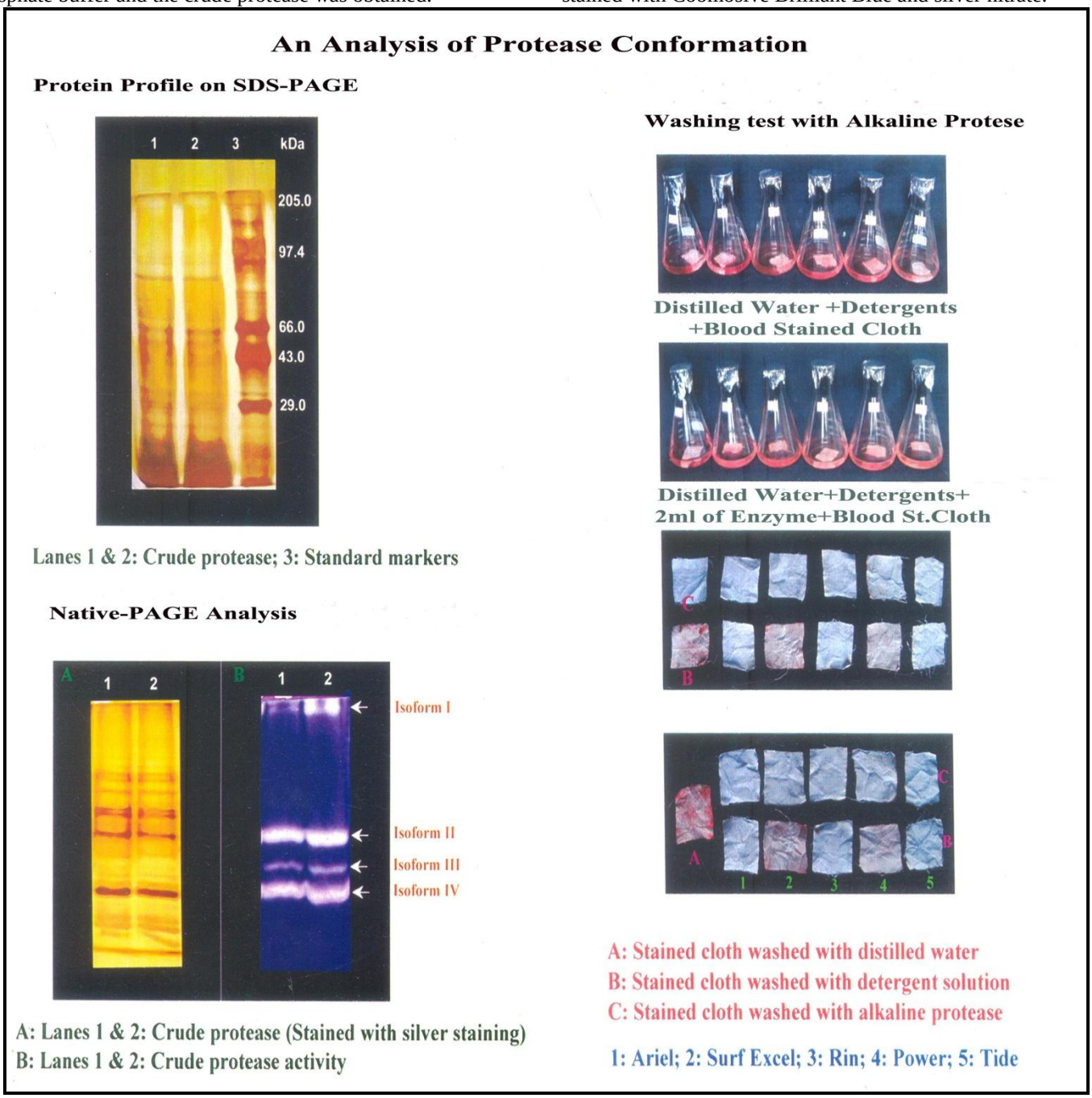

Figure 1: An analysis of Protease conformation

IV. DISCUSSION

Actinomycetes strain isolated from marine sediment were identified as they produce alkaline protease. The proteolytic activity of the marine streptomyces sp.MML1614 was detected, in which the casein and gelatin forms a clear zone around the colonies.Also streptomyces lividans 1326 produces significant clear zone when it was grown on milk agar plates. The formation of clear hydrolytic zone indicates that the marine strain of

streptomyces M11ViLL1614 produce extracellular protease. (Dekleva *et al*. 1985).

Extracellular production of protease is influenced by various physical factors, such as media composition, pH, temperature and incubation time The marine streptomyces sp.NIIvIL 1614 was exhibite good growth and high protease production at pH 7, and 28°C. Maximum growth of streptomyces sp.NIIvIL 1614 was observed in in 40°C. It had favored the maximum rate of enzyme production. (Nehete *et al*., 1985)

Secretion of alkaline protease dependents on the available sources of carbon and nitrogen in the medium.Various nitrogen sources, including amino acids are found to repress the production of enzyme at certain concentrations. The marine streptomyces sp. NUvIL1614 produce high amount of protease when glucose was used as carbon source (Gibb and Strohl, 1988) Nitrogen source plays a key role in protease production.Protease production in streptomyces sp.MML1614 was increased with the addition of peptone (upto 2%) but beyond a certain level the production rate of protease was repressed. (Kanekar *et al*.,2002)
In general,Liquid-state Fermentation is more convenient than submerged cultivation. Hence, it is feasible to apply the solid-state cultivation for producing agricultural by-products. In LSF, microorganisms secrete the necessary enzymes to degrade the available substrates to fulfill their nutritional requirements. (Tunga *et al*., 1998) Protease production medium (PPM) was prepared to inoculate actinomycetes. Then the enzyme was collected on 6^{th} day by filtring the medium

Agro-industrial wastes/substrates (defatted soybean cake, gram bran. wheat bran, rice bran, banana waste, etc) can de used for the production of Protease enzyme. Solid state fermentation is a simple process and requires less pre-processing energy than submerged fermentation. (SMF) Other advantages include superior productivity, low waste water Management, and improved product recovery (Tunga et *al*., 1998).

As our protease produced by marine Actinomycetes, it can able to withstand wide range of pH and temperature. Also it shows the compatibility to various commercial detergents; that it can be used as an additive detergent. These additive proteases improve the cleaning performance of the detergent. Additions of protease enzyme in surf excel results in increased blood stain removal ability of it. Protease plays a vital role in dehairing process of leather industries.

V. CONCLUSION

Protease plays a vital role in various industrial applications, and the demand for protease is increasing day-by-day. Therefore, it is expected that hyperactive strains will emerge and the enzymes produced by these Actinomycetes could be used in different industries. In order to meet the ever-growing demand for this enzyme made us to search for new protease producing microorganism.

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