Research Paper Microbiology

Isolation, Identification, Characterisation Of Bacterial Species From Keratinase Producing Microorganism

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ABSTRACT

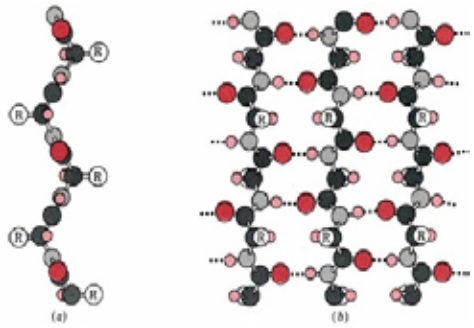
Bacterial keratinase are of particular interest, because of their action on insoluble keratin substrate & its broad range of protein substrate. These enzyme have been studied for dehairing processes in the leather industry and hydrolysis of feather keratin, it's a by-product generated in huge amount by the poultry industry. Discarded feather meal through thermal processing resulting in a low nutritional value product. Feather hydrolysate produced by bacterial keratinase has been used as additives for animal feed. In addition keratin hydrolysate have potential use as organic fertilizer production of edible films .Cultivation condition are essential in successful production of an enzyme and optimization of parameter such as pH,temperature and media composition is important in developing the cultivation process. The work was designated with the few objectives include the screen for extracellular keratinase with bacterial species from poultry waste, characterization of the enzyme, view the substrate in SEM

Keywords :Feather waste, keratin, keratin production, Bacillus organism, SEM, SDS-PAGE.

Introduction:

Keratins occur in nature mainly in the form of hair and nail. Keratin is the most structural protein and is components of epidermal and skeletal tissue. A disulphide and hydrogen bound makes keratin a stable protein resistance to proteolytic hydrolysis (Arai et al., 1996; Jones et al., 1999). Keratin like other protein is made of a long string of various amino acids, which fold into a final 3-dimential form. Alpha helix and Beta – sheet consists of flat structure. Basically keratin is divided into two categories, the soft and hard. Outer layer of epidermis of the skin is an example of soft keratin. Hair, Wool, Feather through classified as hard keratin. Depending upon the percentage of sulphur content, the Hard and Soft keratin are classified. Hard keratin about 5 % high and as low as 1% in soft keratin of Sulphur content. Keratin contains 22 amino acid especially in the helical region of their structure. Important amino acid present in keratin are Cysteine, Arginine, Serine, and Glycine.

Beta keratin



Feather waste product annually, which consists of approximately 90% keratin(Santos et al.,1996).A current value added use of feather is the conversion to feather meal, a digestible dietary protein for animal feed.

Feather waste generated in large quantities as a By-product of commercial poultry processing plant. The degradation of keratinous material is important in medical, agricultural etc. (Shih and Williams et al., 1990; Shih 1993, Matsumoto 1996). For clearing observation in the sewage system during waste treatment and eco-friendly dehairing process in leather industry (William et al., 1992). The use of these enzyme as an alternative to dehairing catalyze in leather industry in slow release nitrogen fertilizer, cosmetics and bio-degradation films (Rju et al., 1999 ; Riffel et al., 2003a ; Wiegan et al.,1999 ; Choi and Nelson .,1996 ; Schrooyen et al., 2001).Basically keratinous protein are insoluble and resistant to degradation by common proteolytic enzyme trysin, pepsin, papain because of their high degree of cross-linking by disulphide bound, hydrogen bounding and hydrophobic interaction. Keratin hydrolysate use potential application in leather tanning industry. Keratinases have been reported from several species of Bacteria and Fungi (friedrich et al., 1999).

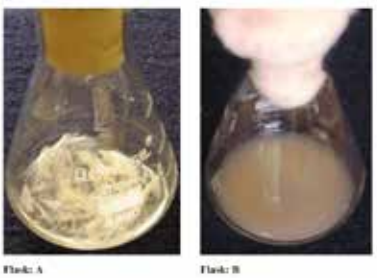
In this article, the description about the isolation of a keratinolytic strain followed by isolation, identification and purification of alkaline keratinase able to accomplish the degradation of chicken feather.

Material and Method:-

i) Isolation of Microorganism:

Soil sample (Soil and feather) were taken from the natural composting in the poultry shop at Coimbatore City (Tamil Nadu).All the sample (500gm of each)were transported in a plastic bag to the laboratory (Ilham and Mohamed.,2003).Serial dilution from each sample were prepared by adding 1gm of the soil sample to the 9ml of distilled water. The diluted water plated on Nutrient Agar medium and incubated at 35C for 24hour.The appeared colonies were checked for spore presence and streaked on agar slant for further characterization.

Extracellular keratinase producing bacterial species from Poultry waste



ii) Identification of feather degrading bacteria especially in chicken feather:

From the isolated colonies, the culture is inoculated by streaking method on Casein agar plates. To identify the proteases exhibiting bacteria and theses proteases exhibiting bacterial colonies were transferred onto fresh casein agar plate for confirmation result. Organisms showing proteases activity was further checked for keratinases.

Spread plate techinuque

Casein Agar Plate

iii) Identification of microorganism using trace salts:

Colonies showing keratinases activity were grown in a medium containing trace elements feather as carbon and nitrogen source (NaCl-5gm, K2PO4-.4gm, Feather-10 gm, Distilled water-1L).The Culture was incubated for 15 days at 30C.

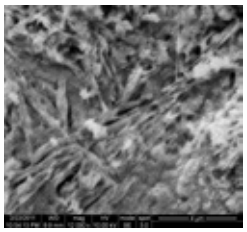
Different type microbes

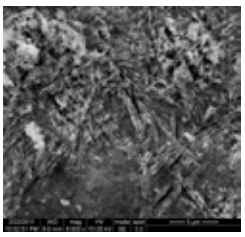
iv) Protein Assay:
The protein content of the enzyme preparation was estimated by the Lowry method (Lowry et al., 1951).

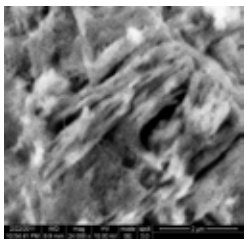
v) Screening method:
The bacterium used in this study was patented strain of Bacillus sp., isolated in our laboratory. All culture condition and the feather culture medium were as previous described. The medium was prepared and pH 8 is maintained. The feather were washed, dried, and cut into smaller pieces (2-3cm) prior to being added to the medium. The medium was sterilized by autoclaving. The bacterium was cultured in a test tube containing 10ml of culture medium. After 20 days incubation, 10ml of the culture medium was transferred to 3l conical flask containing 1l of the medium. After 7 days of incubation the medium was collected for keratinase purification. All incubation was done at 35C with shaking at 150rpm in a controlled environment shaker.

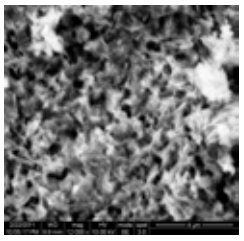
vi) Keratinase assay:
Keratinase activity determined by modified method (Letourneau et al., 1998) using keratin azure (Sigma chemical, USA) as substrate. The Keratin azure was suspended in Carbonate buffer (10mM, pH-10) at concentration of 4mg/ml. The reaction mixture contained 1ml of enzyme and 1ml of keratin azure suspentation. The sample was inoculated at 37C, 300rpm for 1hour.After incubation, the mixture was kept in ice for 15 minutes followed by centrifugation at 5000rpm for 15 minutes to remove unutilized substrate. The supernatant was spectrophotometrically measured for the release of the azodye at 595nm.Acontrol was kept with enzyme and buffer without substrate.

vii) Electron microscopic examination (SEM):
Feather sample taken from inoculated broth and control taken from un-inoculated broth. These two samples are examined with the SEM for their degradation process.









viii) Purification of enzyme:
Culture broth was cooling centrifuge (600gX for 15 minutes) to remove cell and debris and the supernatant was collected. The keratinase purification was done by using the salting out with ammonium sulfate was added to the supernatant with a solution (30–90%) and Acetone (30-90%) fractions.

ix) SDS-PAGE:
Sodium dodecyl sulphate polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970) using 12% cross-linked polyacrylamide gel. Silver staining was carried out to visualize protein bands on the gel (Morrissely, 1981).From these protein bands the molecular weight of this protein is compared with the help of standard molecular marker (220-14 KDa) {Bio-rad-USA}.

x) Characteristic of crude enzyme :
Effect of pH:
The optimum pH of the crude enzyme by dissolving the keratin at various buffers. The acetate buffer-0.05mol/l pH- 8.5; Carbonate buffer-5.5 pH; Phosphate buffer-pH 8.The enzyme was incubated at the pH 8.5. The incubation enzyme solution of pH ranges from 4-9.

Effect or Temperature:
According to the various pH as well as various temperature has been done. The temperature ranging from 45 – 90C. With the optimum pH of the crude enzyme as constant the

optimum temperature was found by incubating the enzyme with substrate.

Effect of metal ions:
The varying concentration of metal ions includes the 1mM Phenanthroline, EDTA in 0.05mol/l PO4 buffer (pH- 7) at 37C for 30 minutes. The enzyme activity was of Zn2+,Mg2+,Hg2+ at (1-3mM) concentration for 1 hour at 37C.

Result:
Isolation of microorganism:
Feather degrading microorganism was isolated from soil sample in near poultry farms by serial dilution, spread plate method. The master plate contained microorganism exhibited distinct morphology and its streaked into casein agar and nutrient agar medium. One type of organism showing .

Keratinase activity on intact chicken feather:
The degradation of intact feather by Bacillus sp was achieved in 96 hours of incubation in trace salts medium at 30C.In the present study Bacillus sp was also to produce Keratinous in feather meal, medium and degrade the whole feather in mineral salt medium. The ability of the Bacillus sp., to grow and produce approximately level of keratinase using feather as a substrate could often produce tremendous potential for the development of BT method for the hydrolysis of feather the potential application of feather is a cheap and readily available substrate, can be used for the production of keratinase at the industrial level. Currently, over 30% of the total Industrial enzyme market is accounted by proteases used for detergent, leather tanning and food production. Use of feather, a cheap and readily available substrate, could result in a substantial reduction in the lost of enzyme production.

Screening and feather degradation:
The isolated and identified Bacillus sp.,,was inoculated into the feather meal broth which contained feather and incubated in 37C for 10 days incubation in incubator.

Characterization crude enzyme:
The range pH 4-9. The effect of enzyme activity was determined at the range 8.5. At this pH only the enzyme activity shows high level enzyme stability/production.

The temperature range from 45 to 90C. The high level of enzyme activity/stability was carried out the enzyme solution at temperature range of 80C.

In metal ions the enzyme activity shows high level of variation. Especially in the Zn2+ shows high level enzyme activity as compared to other metal ions. This is determined by the lysis of feather powder.

SDS-PAGE:
After the purification of enzyme was done by using the ammonium sulphate precipitation and acetone precipitation. The purified enzyme was collected for the analysis of molecular weight using SDS-PAGE. These fractions are loaded on to SDS-PAGE set up. The molecular weight of the purified faction can be determined and clearly visualize in the silver staining method. The molecular weight of the purified enzyme fraction is about approximately 45,000 Dal.

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