Volume : 2 | Issue : 2 | Feb 2013 • ISSN No 2277 - 8160

Research Paper | Microbiology

Isolation, Identification, Characterization of Bacillus Cereus 4080 LBK Producing the Keratinase Enzyme

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ABSTRACT

The aim of this study to isolate the keratinase producing bacteria from poultry farm / dumping soil. Feather dumping soil was collected from several area include the Coimbatore and pollachi (poultry shop).All the Eighteen isolate were subjected for primary screening on milk casein agar plate. In that, five isolate showing a high zone of hydrolysis in feather meal agar plate. These plates were again subjected to keratinase activity assay and identified the spectrophotometric assay by the keratinase azure as a substrate. The feather degradation process can be identified by the Transmission electron microscope (TEM) for specific two Bacillus sp. (one it shows high zone of clearness in Feather meal agar plate i.e. a Bacillus cereus 4080 LBK). The keratin hydrolysis used for animal feed, leather industry, organic fertilizer for the edible films etc.

Keywords: Chicken Feather, Keratin, Keratin Azure, Bacillus Cereus 4080 Lbk...

INTRODUCTION:

Basically keratin, a fibrous protein forming main structural constituent of feather, hair, wool, horn, hoof, etc. (Arai et.al 1996; Jones et.al., 1999).

Keratin was first coined by Kuhn 1878 and it was first analyzed by Per sox in 1883. Insulin protein is the sequence of amino acid and it's demonstrated by Sanger. (www.keratin.com).

Keratinase belong to the group of serine hydrolyses that are capable of degrading keratin. The keratin is a fibrous and insoluble structural protein extensively crosses linked with disulphide, hydrogen and hydrophobic bounds. (P.Anbu and S.C.B Gopinath et.al.,2007). Chicken feather are approximately 91% protein, 1% of other feather. The amino acid is characterized by a high cysteine, which may vary between 2 to 18wt % (S.C.Mishra et.al. 2009). Keratinolytic protease could play an important part/application in enzymatic improvement of feather meal, Production of amino acid / peptide from high molecular weight substrate and in the leather industry (P.Anbu, S.C.B Gopinth et.al.2005). Compared to the Alpha helix and Beta helix of keratin. The chicken feather mostly Beta helix structure of the keratin. Keratinase enzyme that have been purified from different microorganism to date, such as fungi (Muhsin and Aubaid.,2000; Brouta et.al.,2001; Farag and Hassan .,2004;Gradisar et.al.,2005); bacteria(Cheng et.al.,1995; Bernal et.al.,2006a) and all act as a protinase and high level of activity on insoluble keratin.

Bacterial colonies are known are capable of degrading feather. These Bacterial strain produce the enzyme by using the carbon, nitrogen, sulfur for the energy source (Savithri and G.Joshi et.al. 2007).

In this article, we describe about the screening and characterization of keratin using the chicken feather as a substrate.

CHARACTERIZATION OF CRUDE ENZYME:

i) Effect of pH:-

The optimum pH of the crude enzyme is dissolving the keratin at various buffer .05mol/L is prepared for acetate, carbonate, phosphate buffer at varying pH level. The pH of the buffer varying according to the dilution, its include 8.5, 6, 8. The enzyme activity is determined by the standard graph.

ii) Effect of temperature:-

Similar to the pH, the temperature range is varying from 4-700C. With the help of enzyme (substrate) the high level of enzyme activity at the specific temperature range can be determined by using the standard graph.

iii) Effect of Thermal activity:-

The temperature stability and enzyme activity are mixture thoroughly and it's incubated at the different temperature range about 25-800C by using carbonate buffer as a solvent. It's done by using at different pH range between 8.5 & 12, Keratin azure as a substrate/solute. Before preceding the thermal activity, the solvent reaction mixture is pre-inoculated about 600C for 1 hour. Here, we can measure the residual activity/thermal activity in the spectrophotometer.

PURIFICATION OF THE ENZYME:

The keratinase enzyme can be purified by using several techniques. The partial purification can be performed by acetone, ammonium sulfate and complete purification can do by chromatography technique (paper).

i)Partial Purification:-

The culture broth of the Bacillus cereus was taken. Then, the broth get centrifuged at (600gX15 minutes) to remove the cell and debris. The supernatant was taken for further purification step. The collected supernatant was salting out with ammonium sulphate (30-90 %) and the acetone (30 – 80 %) fraction.

ii) Paper Chromatography:-

After completing with that purification, crystalline from of particles is taken for further purification process. For that, Whatmann's no: 1 filter paper is taken and its length is about 7X5cm. The solvent will be prepared by using the 4:3:1 concentration of n-butanol, acetic acid, water. The solvent poured in the chromatographic chamber , the sample containing filter paper is dipped in the solvent. The bands will be viewed after 2 hours incubation.

iii) 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}.

Result:

The soil sample was taken and it's diluted in the sterile saline. The nutrient agar was prepared, sterilized and it's inoculated the soil sample. The plate incubated at 370C for 24 hours. The organism is all again inoculated in the CAM. Those organism are able to produce the casein, again these are all inoculated into milk agar/CAM. All positive culture (18) can be used for further analysis.

In the 18 culture, the Bacillus cereus shows the highly potent keratin activity in both primary and secondary screening technique. I the primary screening the Bacillus cereus produce high zone of clearness in CAM (Casein agar medium) an secondary screening, the Bacillus cereus utilize the feather for fully degradation. In that, the feather acts as a carbon, nitrogen source for their degradation. Basically, the feather keratinase having a high disulfide bond, its make very hard to degrade the feather.

In keratinase activity assay, the enzyme can be determined by the absorbance in the spectrometer. As a result, the azo dye is releasing at the level of 595nm.The nit of absorbance in the crude enzyme value is about 10.75 U/ml.

The characteristic of the crude enzyme is classify /effect it's include the pH, temperature, thermal activity. In the effect of pH by using different type of buffer. The specific Ph (phosphate –pH8) shows high level of enzyme activity is about 468.5.According to the pH shows that best result. Similarly the specific temperature range in enzyme activity is about 550C. In that the result is as 635.25 KU/ml can be measured in spectrometer. To calculate the thermal stability, the reaction mixture

and substrate can be measured in the spectrometer at the 595nm. In this, the releasing of azo dye is high at the temperature 550C , the value is 630KU/ml.

In TEM analysis, the feather will be degraded about 2 µm in size. Indicating that the, B .cereus will utilize the feather as a carbon and nitrogen source.

In MOLDI-TOFF-MS (LINEAR) analysis, the specific organism is B .cereus 4080LBK shows the highest value (2.045) and it's ranked in first position.

In partial purification step, by using the ammonium sulfate (30-90%) and acetone (30-80%), the keratinase enzyme will be presented as a white crystalline form.

In the complete purification process, the paper chromatography and SDS are used. With the help of the solvent, the solute is passed over the filter. After the 2 hours incubation, then filter gets dried. Then the filter paper is sprayed with nin-hydrine, and then the bands will appear as yellow color bands. 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 42,000 K Dal.

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