Vol III Issue VII Jan 2014

Impact Factor : 1. 9508(UIF)

ISSN No :2231-5063

International Multidisciplinary Research Journal

Golden Research Thoughts

IMPACT FACTOR : 1. 9508(UIF)

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RNI MAHMUL/2011/38595 **ISSN No.2231-5063**

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Golden Research Thoughts
ISSN 2231-5063
Impact Factor : 1.9508(UIF)
Volume-3 | Issue-7 | Jan-2014
Available online at www.aygrt.isrj.net

BIODEGRADATION OF FEATHERS BY THE MICROORGANISMS PRESENT IN THE POULTRY WASTES AND ENHANCE ITS ACTIVITY BY MUTATION

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Abstract:-Feathers are composed of over 90% protein and produced in large amount of solid waste and deposited in aquatic environment leads to various diseases to human and aquatic animals. Microbial degradation using wild and mutant strains of *B. cereus* and *P. aeruginosa* is an effective process to remediate feather waste. These organisms were widely found in rotted feather and soil of poultry waste. The degradation rate was also high mutant strains of *B. cereus* compared to *P. aeruginosa* and wild strains of both. SDS-PAGE analysis proved that the mutant strains had longer polypeptide chains of keratinase enzyme than wild type. Therefore it is an effective method of degradation and the end product also acts as an animal meal.

Keywords: *Bacillus cereus*, Feather, Keratin, Mutant, *Pseudomonas aeruoginosa.*

INTRODUCTION

Feathers and other poultry waste is one of the major problems of causing disease to human, animals and aquatic forms. Feathers are composed of over 90% keratin and produced in large amounts as a waste by poultry processing worldwide (Onifade *et al*., 1998; Cheng-gang Cai *et al*., 2008) and it cannot be degraded easily because they are structural proteins contain large quantities of the sulfur – containing amino acids particularly cysteine, it linked by disulfide bridge form tough fibrous matrix.

Traditional ways of degrading feathers such as alkali hydrolysis and stream pressure cooking may not only destroy the amino acids but also consumes large amounts of energy. Therefore biodegradation of feathers by keratinase from microorganisms provide a viable alternate. Bacteria (El Refai *et al*., 2005), fungi (Friedrich *et al*., 2005) and Actinomycetes (Gusterova *et al*., 2005) have previously been shown to be able to produce feather-degrading keratinases but the amount of this enzyme is very low. Hence the large scale fermentation for the higher yield of these enzymes and the purification process become difficult and costlier. Almost all keratinases are inducible and different keratin-containing materials such as feather, hair and wool can be used as substrates for keratinases production (Gupta and Ramnani, 2006).

There are many different methods are carried out for the mutation process such as chemical and physical methods to improve the yield of enzymes. In this present study, we prepared cost effective method for degradation proteins present in the feathers. We have compared wild and mutant strains of *Bacillus cereus* and *Pseudomonas aeruoginosa* keratinase activity against poultry waste.

MATERIALS AND METHODS

Sample Collection

Rotted feather and soil were collected from Suguna poultry plant, Namakkal. The samples were shaken in 0.85% (W/V) NaCl solution for 30 minutes. The solution was kept in the shaker incubator for enhancing the growth of dominant microbes present in the samples.

Isolation and Identification

Samples (100µl) were taken and spread over the plates of nutrient agar individually. Then it was incubated at 37°C for 48 hours. After that the individual colonies were streaked on LB agar plate and incubated at 37°C for 24 hours. Pure cultures of microbial colonies were taken and perform gram staining and biochemical test (Indole, MR, VP, Urease, TSI, Motility test) for identification.

EXPERIMENTAL DESIGN

Preparation of Feather powder

Feathers were collected, washed with tap water and distilled water for 2 to 3 times. Prior to autoclaving air dry the feathers. After autoclave dried in hot air oven and care was taken that the temperature should not exceed 40°C. The dried feathers were grinded into powder form. It was stored for further process.

Preparation of Feather Meal Agar

For preparing 1000 ml media, all the components such as NaCl (0.5g) $MgSO_4$ (0.1g), KH_2PO_4 (0.7g), K_2PO_4 (1.4g), Feather powder (10g), Agar (15g) were added in the order listed by stirring continuously. It was autoclaved by adding 100ml of the medium in each conical flask, then it was used for further purpose.

Preparation of Mutant Strains

Selected strains were inoculated into LB agar plates. It was then placed under physical ultra violet radiations for 1 to 2 minutes and incubated at 37°C for 24 hours. Care was taken for UV exposure time on strains. Mutant and wild strains of *B. cereus* and *P. aeruginosa* were inoculated into feather meal agar and incubate at 37°C for 3 days.

Isolation of Keratinase

Ten ml of the wild and mutant cultures were taken and centrifuged at 10000 rpm for 15 minutes at 4°C. The pellet was homogenized in 5 ml phosphate buffer at pH 7.0 in a sonicator, it was again centrifuged. The supernatant were taken for enzyme analysis.

Dialysis

Dialysis is mainly carried out to purify the exact enzyme and find its property. Two dialysis bags measuring 3 cms were taken and tied with thread at one end of the bag, now it is poured in a boiling water bath to open the bag. The bags were filled with crude enzyme extracts. The dialysis bags were kept in 500ml of phosphate buffered saline in a beaker. The purified enzymes were present in the bags it was taken and stored in vials at 4°C.

Analysis

Lowry's protocol was followed to estimate the enzymes using BSA as standard in the concentration of 10 to 100 µg/ml (Lowry *et al*., 1951). Qualitative determinations of enzymes were done by using TLC, Leucin was used as standard. Iso propyl alcohol and Ethanol in the ratio of 80:20 was used as mobile phase and Rf value was calculated. SDS – PAGE were performed to determine the molecular weight of enzymes isolated from wild and mutant strains.

RESULTS AND DISCUSSIONS

The microscopical and biochemical tests proved the presence of *B. cereus* and *P. aeruginosa* in the poultry samples. TLC method showed the 0.86 Rf value with Dark purple colour for Leucin. In the case of *B. cereus* and *P. aeruginosa*, 0.83 and 0.85 was observed, which relatively equal to that of standard, so the protein sample must be keratinase.

Estimation of Enzymes by Lowry's Method

The mutant strains of *B. cereus* and *P. aeruginosa* showed maximum production of keratinase enzyme compared than wild type. Mutant *B. Cereus* and *P. aeruginosa* produced 112 µg/ml and 145µg/ml whereas the wild strains synthesis less amount of enzymes such as 89µg/ml and 136µg/ml.

Comparison of Feather Degradation

The mutant bacterial strain *B. cereus* in liquid culture with feather showed degradation in log phase. The maximum activity of *B. cereus* can be viewed in the 3rd day of inoculation and it gradually decrease on the 4th day. Enzyme production is gradually decreased in death phase. The complete process of feather degradation in the feather meal agar and the comparison study is shown in Fig.1 The degradation of feather was carried out by the utilization of keratin present in the feathers.

The mutant *P. aeruginosa* plays a vital role in the degradation of feathers. It produced certain extra cellular enzymes such as protease and keratinase. This enzyme produces in higher content when compared to wild strain. The volume of enzyme was increased on 4th day. Fig. 2 showed the degradation of keratin by the keratinolytic organisms.

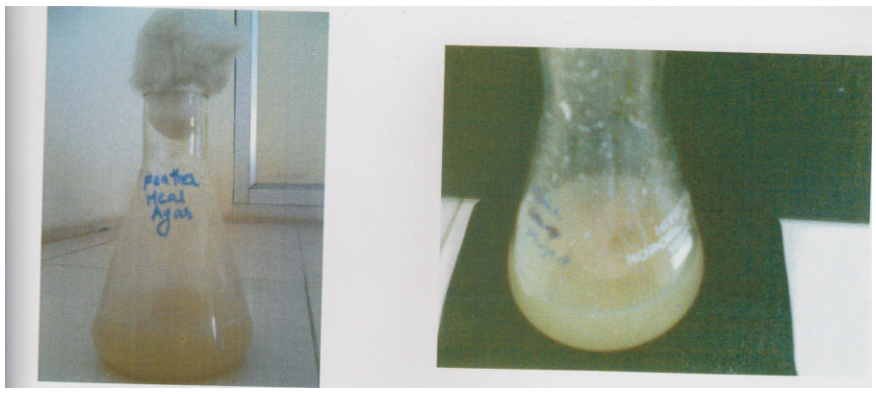

Fig. 1 Degradation of Feathers by *B. cereus* Mutant Strains

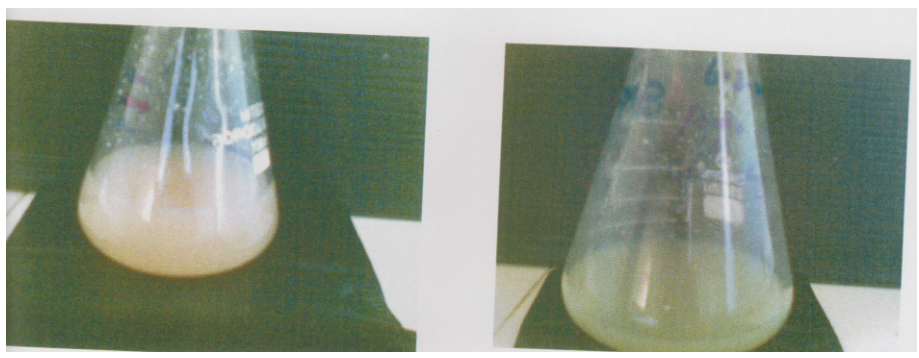

Fig. 2 Degradation of Feathers by *P. aeruoginosa* Mutant Strains

Molecular Characterization

SDS-PAGE studies revealed the molecular weight of wild and mutant strains. The wild *B.cereus* and *P.aeruginosa* produced 29KDa of keratinase whereas mutant produced larger size such as 56 KDa of enzymes. Fig. 3 showed Kearatinase enzyme separated in SDS-PAGE. Ionata et al., 2006 purified the keratinase enzyme (28.7 KDa) from culture supernatant. A keratinase Q1 enzyme was purified from *Chryseobacterium* sp kr6 culture by Phenyl Sepharose and Superos by Riffel, et al., 2007.

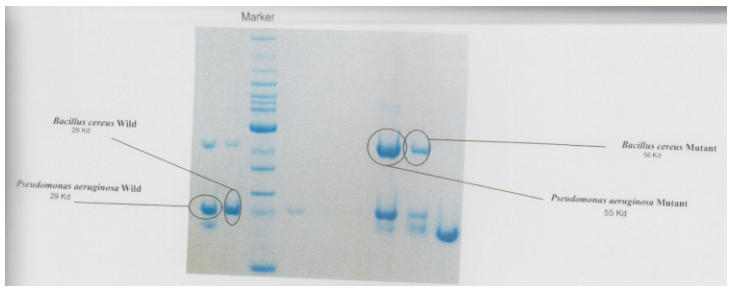

Fig. 3 SDS with Marker and the Keratinase

CONCLUSION

Feather degradation is one of the important processes to secure the environment from disease and pollution. Feather and other poultry waste were being degraded by the extra cellular enzymes which are present in the poultry waste. We enhance its activity by UV radiations it produce this enzyme in higher quantity. The enhanced activity which is produced by mutation does not cause any harm to the environment. This is one of the economical way of degradation and will benefit a lot to the feature generation.

ACKNOWLEDGEMENT

We thank The Principal and Head, Biotechnology Department, New Prince Shri Bhavani Arts and Science College, Chennai and Suguna Poultry Plant, Nammakal for collection of samples to carry out this research work.

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