Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2011, 1 (2):56-63

ISSN: 2248 –9215

Feather Waste biodegradation as a source of Amino acids

Avinash Srivastava, Anshul Sharma and Vuppu Suneetha*

School of Biosciences and Technology, VIT University, Vellore (TN) India

---

ABSTRACT

Feathers are composed of beta keratin protein. A Bacillus strain SAA5 has been isolated from feather dumping site and used for evaluating the keratin substrate as a source of amino acids along with the production of alkaline protease. Fermentation using feather as a substrate was carried out on minimal salt media for 7 days which resulted in almost complete degradation of feather. The optimum conditions for keratinase production were temperature 30˚C, pH 8.5 and initial substrate concentration 1%. An increase in the pH of medium was also reported during the period of fermentation. Rates of feather degradation and enzyme production maximized during the logarithmic phase of growth. Maximum enzyme activity was found to be 6.5 IU/ml on fifth day. Concentrations of total amino acids, cysteine, serine, and methionine were continuously monitored in the fermentation broth and reached to a maximum of 1.2mg/ml, 46µg/ml, 107µg/ml and 17.52µg/ml respectively.

Keywords: *Feathers, keratinase, Cysteine, Methionine, Serine.*

INTRODUCTION

A total of 5-7 percent weight of mature chicken comprises of feathers. Feathers are composed of beta keratin [9] which is an insoluble protein and has a stable rigid structure because of several cross linking disulfide bonds involving cysteine. Keratin is also very rich in amino acids like Leucine and Serine. A number of feather degrading species of bacteria, actinomycetes and fungi have been used for the production of keratinase enzyme in submerged [17, 2] as well as in solid state fermentation [12]. Most of keratinase enzymes from microbial sources are extracellular and inducible by keratin waste. There are several reports indicating the enzyme to be an alkaline protease [1]. The enzyme finds important application in leather industry [4]. In current practices

raw feathers are being utilized for animal feed through a high energy input processes. The feather meal so prepared has very low digestibility and thus availability of amino acids is low. Nutritional enhancement can be achieved by releasing the free amino acids from the poorly digestible protein using feather degrading bacteria [18]. This report describes the isolation and characterization of a feather degrading bacterium. The optimum temperature, pH and initial substrate concentration for achieving maximum keratinolytic activity and feather degradation were determined.

MATERIALS AND METHODS

Isolation and characterization*:* Soil samples were collected from the feather dumping sites near Katpadi (Vellore, India). 1 gm of sample was suspended in 10 ml of sterilized water and after diluting to an extent of 10^{-8}, an aliquot of 50 µl was spread on the Luria Bertani agar plate. For screening of feather degrading bacteria, hair baiting technique [13] has been used; instead of hairs sterilized feathers were used. Single colonies were isolated and then stored on LB agar slants at 4˚C. These colonies were screened for their ability to degrade the feather, directly on the same media used for fermentation. Extent of feather degradation in the fermentation media was observed for each culture. The culture which was showing the maximum extent of feather degradation was used for further studies. Morphological and physiological studies were performed as per Bergey's Manual of Systematic Bacteriology [7] to characterize the isolated bacterium.

Substrate: Chicken feathers collected from nearby poultry farm were used as substrate. Feathers were washed with distilled water and subsequently dried in hot air oven at 50˚C. After this pre treatment was done by keeping these feathers in chloroform: methanol (1:1) solution for 48 hrs. These feathers were again dried at 40˚C and then stored at 4˚C for further use.

Media Composition: Media that was used for feather fermentation consisted of 0.5g NaCl, 0.3l K2HPO4, 0.4 g KH2PO4, 10gm feather per litre of distilled water [15]. pH was adjusted to 7.5 using 0.1 N NaOH and 0.1 N HCl.

Inoculum: 50 ml of LB broth was inoculated using bacterial slants. After an overnight incubation, 5 ml of this broth was taken and centrifuged at 8000 rpm for 10 minutes to pellet down the bacteria. This pellet was resuspended in 5ml of sterile water which was subsequently used for inoculating 100 ml of media.

Fermentation Conditions: The fermentation was carried out in 500ml Erlenmeyer flasks with 100 ml of media. The culture was incubated at 180 rpm, 30˚C for 7 days.

Bacterial Growth: Bacterial growth was determined by plate count methods on LB agar plates [10].

Feather Degradation: Residual feathers were harvested from the fermentation media by filtering it over whatman filter paper 3. The harvested feathers were kept in hot air oven at 50˚C until weight stabilized to constant value. The difference between the weight of residual feather

obtained from the control and that of inoculated media has been used as measure of feather degradation. Degradation was expressed in percentage.

Keratinase activity: Samples were regularly harvested from the flask containing inoculated media. Biomass was removed by centrifugation at 10,000 rpm for 10 min. The supernatant obtained was used for determination of enzyme activity. 40 µl of supernatant and 4 mg of feather substrate were added to the 760µl of Tris-HCl buffer (pH 7.5). Reaction was carried out at 37˚C for 1 hr after which assay mixture was left on the ice for 10 minutes to stop the reaction. Residual substrate was filtered out and absorbance of the filtrate was measured at 280nm wavelength. An increase in absorbance value by 0.1 units as compared to the control was taken as 1 unit of keratinolytic enzyme [16].

Amino acids: Total amino acid content was estimated at regular interval in fermentation broth by Ninhydrin method [11] using Leucine as standard. The concentration of amino acids like Cysteine [14], Serine [5] and Methionine [6] were also measured in fermentation broth at regular intervals.

Effect of pH, Temperature and Substrate concentration: Effect of initial pH (from 6.5 to 9.5), temperature (from 20˚C to 50˚C) and substrate concentration (1gm/100ml to 5gm/100 ml) on the extent of feather degradation and maximum keratinolytic activity was studied.

RESULTS AND DISCUSSION

Isolation and characterization of organism: Six morphologically different colonies were obtained. These strains were designated as SAA1, SAA2, SAA3, SAA4, SAA5 and SAA6. Extent of feather degradation was found maximum in media inoculated with strain SAA5 in comparison to other strains (data not shown). Strain SAA5 was used for further studies.

The results of the morphological and physiological test [7] as per shown in Table 1, indicate that bacterium under study is of family **Bacillaceae and genus Bacillus.**

Table 1 Morphological and Cultural characteristics of Bacillus SAA5

Morphological characteristics	
Form	Rods
Spore	Endospore forming
Gram Stain	Positive
Motility	Positive
Cultural Characteristics	
Strict Anaerobe	Negative
Catalase	Positive
Voges-Proskauer Test	Positive
Starch Hydrolysis	Positive
Nitrate Reduction	Positive
Citrate Utilization	Positive
Acidity on Glucose utilization	Positive
Growth in 7% NaCl	Positive

Feather degradation: The Bacillus strain grew well in minimal salt media containing feather as only carbon source and degraded 91 % of feather in a period of 7 days (Fig. 1). Similar results were reported for other bacterial [18] and fungal strains [8]. The pH of medium was continuously monitored during the course of feather degradation and a gradual increase was observed from an initial value of 7.0 to 8.5 (Fig. 2), which suggests the possible deamination of peptides and amino acids resulting in the production of ammonia. Such alkalization of media was also reported in case of keratinolytic fungi [8].

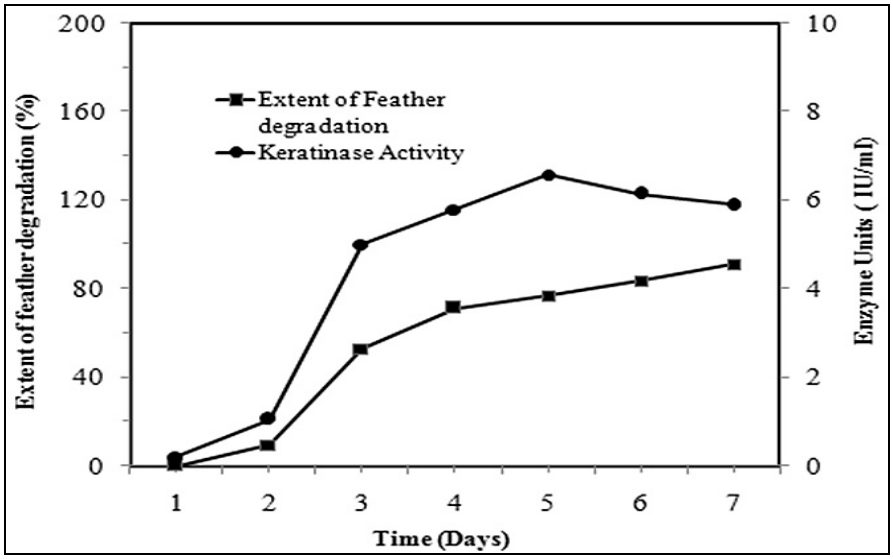

Fig. 1 Time course of feather degradation and keratinase activity

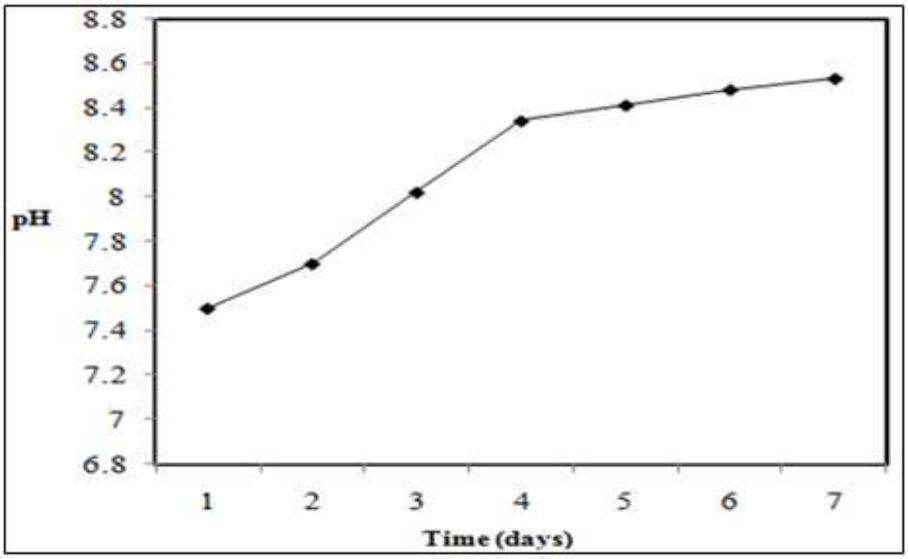

Fig. 2 Increase in pH of medium during the fermentation period

Keratinase activity: The keratinase activity was detected in the fermentation broth just after first day of inoculation. Keratinase activity increases rapidly and reaches to its maximum on fifth day, after that a slow decrease in keratinase activity was observed.

Bacterial growth:

During the fermentation period the bacterial growth was studied and it was observed that it reaches to its maximum of 67 CFU*10^8/ml at the end of the log phase of growth on fifth day. Rates of feather degradation and enzyme production maximized during the logarithmic phase of growth.

Effect of temperature: Temperature has been found to be having profound effect on the extent of feather degradation and bacterial growth. Degradation of feather in the media and the keratinase activity maximize at temperature ranging from 30˚C to 35˚C (Fig 3). Both the extent of feather degradation and enzyme activity declines sharply at high temperature which suggests the mesophilic nature of Bacillus sp. SAA5. Bacillus Mesophilic bacteria can have important implications towards the industrial use because they do not require high energy inputs unlike thermophilic keratin degrading microorganism [3].

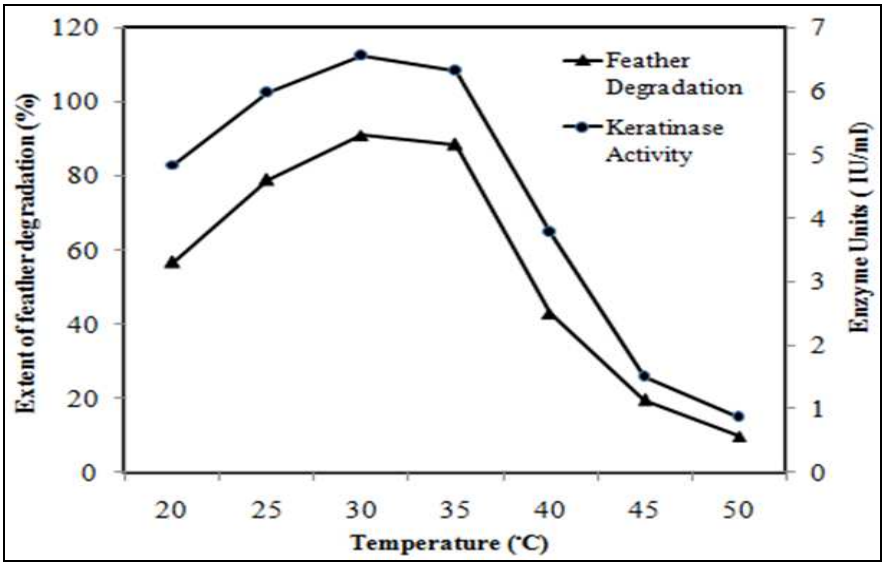

Fig. 3 Effect of Temperature on Feather degradation and Keratinase activity.

Effect of pH: The optimum pH for maximising the feather degradation and enzyme activity was determined by growing Bacillus sp. SAA5 in pH ranging from 6 to 10. At acidic and neutral pH both the enzyme activity and the extent of feather degradation were low but at pH higher than that of 7.5, improvement in the values of these two parameters has been seen. A maximum of 6.87 IU/ml of enzyme activity with about 94% degradation of feather has been reported at pH value of 8.5 (Fig 4). Enzyme produced by Bacillus sp. SAA5 can be classified as alkaline protease because it achieved maximum activity at alkaline pH [1].

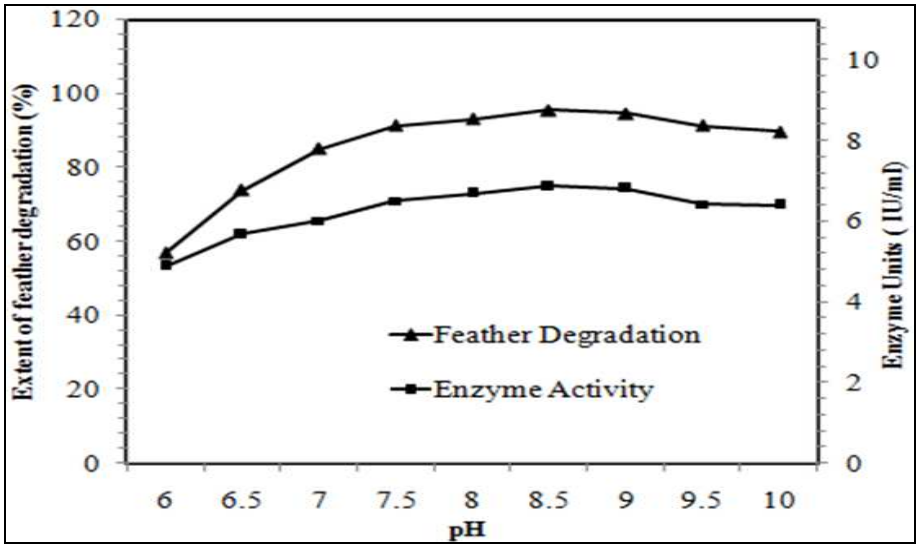

Fig. 4 Effect of pH on the Feather degradation and Keratinase activity

Effect of initial substrate concentration: The initial concentration of feather in the medium affects the extent of feather degradation and keratinase activity (Fig. 5). On increasing the feather concentration the extent of feather degradation decreases because of a decrease in keratinase activity. It indicates that at higher substrate concentration repression of keratinase production can take place. This observation is similar to previous studies which concluded that a low concentration of substrate is optimum for yielding maximum enzyme activity [2].

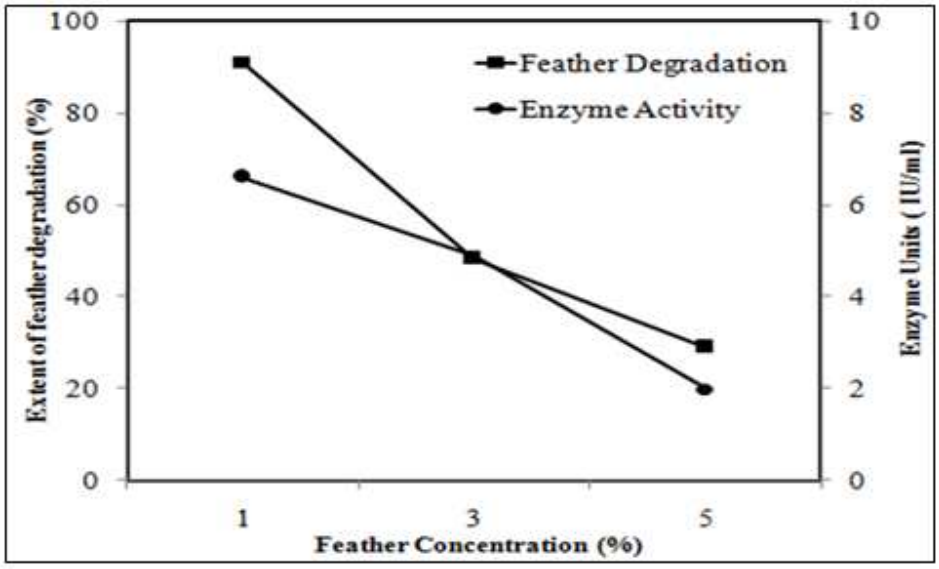

Fig. 5 Effect of Initial concentration of feather on Feather degradation and Keratinase activity.

Amino acid release: Total amino acid content and concentrations of cysteine, serine and methionine were also observed during the fermentation period. Maximum concentrations of

cysteine, serine and methionine observed were 46µg/ml, 107µg/ml and 17.52µg/ml respectively. A maximum concentration of 1.2mg/ml was also detected in case of total amino acid (Fig. 6)

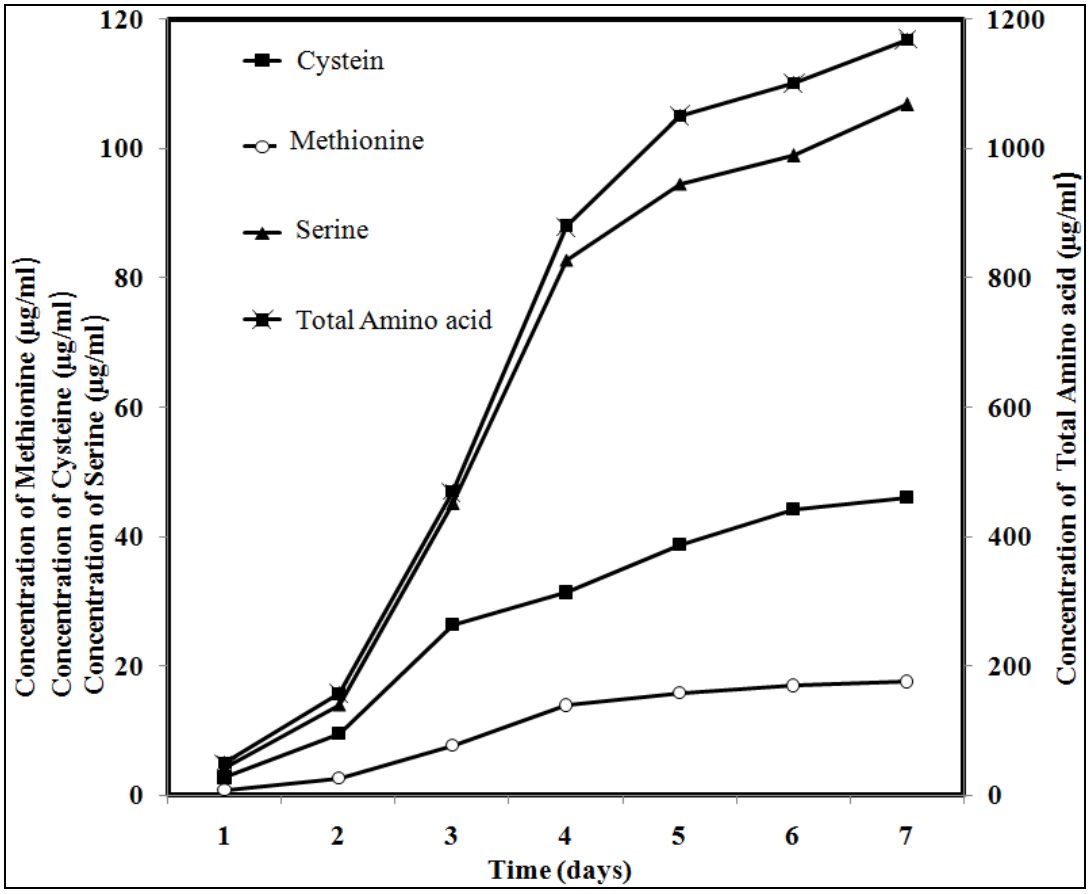

Fig 6 Increase in concentration of Methionine, Cysteine, Serine and Total amino acid during the course of fermentation

Acknowledgement
This study was financially supported by Villgro Innovation foundation, Chennai (India). Authors want to express thanks to VIT University for providing infrastructure support.

REFERENCES

[1] K. Altalo, B.A. Gashe, *Biotechnol. Lett.*, **1993**, 15, 1151–1156.
[2] S.W. Cheng, H.M. Hu, S.W. Shen, H. Takagi, M. Asano, Y.C. Tsai, *Biosci. Biotechnol. Biochem.*, **1995**, 59, 2239–2243.
[3] R.R. Chitte, V.K. Nalwade, S. Dey, *Lett. Appl. Microbiol.*, **1999**, 28, 131–136.
[4] A. Dayanandan, K. Kanagaraj, L. Sounderraj, R. Govindaraju, G.S. Rajkumar, *J. Clean Product.*, **2003**, 11, 533–536.
[5] W.R. Frisell, L.A. Meech, C.G Mackenzie, *J. Biol. Chem.*, **1953**, 203, 709-716.
[6] J.P. Greenstein, M. Wintz; Methionine, Chemistry of the amino acid. Vol. 3, John Wiley & Sons, New York, **1953.**

[7] J.G. Holt, N.R. Krieg, P.H.A. Sneathm, J.T. Staley, S.T. Williams, In: Bergey's Manual of Determinative Bacteriology, *9th edn*,(Williams and Wilkins,Philadelphia,**1994).**
[8] S. Kaul,G. Sumbali, *Mycopathologia.*, **1999**, 146, 19–24.
[9] J.D. Latshaw, N. Musharaf, R. Retrum, *Animal Feed Sci. Technol.*, **1994**, 47, 179–188.
[10] A.A.L. Miles, S.S. Misra, *J. Hygiene.*, **1938**, 38, 732-749.
[11] S. Moore, W.H. Stein, *J. Biol. Chem.*, **1957**, 211, 907–913.
[12] S.K. Rai, R. Konwarh, A.K. Mukherjee, *Biochem Engg. J.*, **2009**, 45, 218-225.
[13] K.C. Raju, U. Neogi, R. Saumya, N. Rajendra Goud, *Int. J. Biol. Chem*., **2007**, 1(3), 174-178.
[14] R. Ramakrishna, P. Siraj, C.S. Prakasha Sashtri, *Curr. Sci.*, **1979**, 48(18), 815–816.
[15] A. Riffel, A. Brandelli, Brazilian., *J. Microbiol.*, **2006**, 37, 395-399.
[16] V Suneetha, V.V. Lakshmi., *J. Enzyme and Microbial Tech*., **2004**. 6, 31-36.
[17] V. Suneetha, Z.A. Khan, In: G. Shukla, A.Varma (Eds), Soil Enzymology. (Springer, Germany, **2011**), 259-268.
[18] C.M. Williams, C.M. Lee, J.D. Garlich, J.C.H. Shih, *Poult. Sci*., **1991**, 70, 85–90.