Research Article

A Potential Beta-Keratin Degrading Bacteria from Vellore Emu Feather Dumped Soil

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ABSTRACT

Keratin is the most abundant structural protein in skin, hair, wool and feathers. Keratins are proteins that form hard fibers and are components of epidermal and skeletal tissues. The aim of this study is to produce pharmaceutically and industrially important amino acids from Emu feather as source using potentially important Keratinolytic bacteria capable of producing Keratinase enzyme. Feather meal is used as sole carbon source and nitrogen source to select the isolated colonies for screening of Keratinase producing bacteria. The isolated colonies were characterized using gram staining. Five colonies were selected for producing Keratinase enzyme. Of these five colonies one showed Keratinolytic activity. The Keratinolytic activity was determined using feather as substrate. The isolated colonies were also tested for Keratinolytic activity using hair, nail and chicken feather as substrates. The optimum pH and temperature was found to be 11 and 37˚C respectively. The enzyme produced was further partially purified with acetone, ethanol and ammonium sulphate. After dialysis Keratinase is further purified with sephadex column packing in chromatography. The new isolated bacterium was used in biotechnological process involving keratin.

Keywords: Feather, Hydrolysis, Keratin, Protease.

INTRODUCTION

The Emu (*Dromaius novaehollandiae)*, is the largest native bird to Australia and the second-largest surviving bird in the world, exceeded only by the ostrich. Most of the Emu grows 5-6 feet tall and weigh 90-130 pounds. Mostly Emus are processed for their meat, fat, hides and its feathers. The smallest emu feather may only be an inch long and very soft while the longest feathers are 18 inches or more and feel like straw. They all have the double plume, or two feathers coming out of one shaft. Comparing with others, emu feathers are much softer because they do not have tiny harbs which making them stiff. They have primary and secondary feather coming out from same shaft.[1,2] Emu Feathers have high oil content and are used for feather dusters on electronic equipment because they do not create static electricity. These emu feathers are made up of keratins. Keratins are insoluble fibrous proteins highly cross-linked with disulfide bridges, hydrogen bonds, and hydrophobic interactions.[3-5] It is becoming a part of solid waste and hence, there is demand for developing biotechnological alternatives for recycling of such wastes. By developing the methods for the hydrolysis of feather into soluble proteins and amino acids could be extremely attractive, and it may provide a cheap and easy method for the production of valuable products like cysteine, methionine and proline.[6] Moreover Keratinolytic enzymes have uses in enzyme research, detergent industry, medical, cosmetic industry, textile manufacturing, and leather industries; they can also be used in prion degradation and as pesticides and production of biodegradable films, glues and foils and also as nitrogenous fertilizer for plants, manufacturing of biodegradable plastics.[7,8] The scope of this paper is to produce amino acids from emu feathers and production of Keratinase enzyme which used for degrading keratin rich products.[9,10]

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used in the study were from Hi Media chemicals, Mumbai and Sigma chemicals, Bangalore.

Isolation of Emu feather degrading organism

Enriched soil was collected from the feather dumping place (near Vellore). From this, one gram of soil was added to 10ml of sterile water and after diluting to an extent of 10-7, an aliquot of 100 µl(0.1ml) was poured on the nutrient agar plate and incubate it at 37'C for 24 hrs. By the next day, sterileemu feathers were placed in the agar plate. By hair baiting technique feather degrading organism is screened.

Substrate pretreatment

Emu feathers were collected from nearby emu farm (near Vellore, Tamilnadu) was used as substrate. These feathers were washed several times with distilled water (approx.: 30-40 times) and dried in sunlight subsequently; Then it is dried in hot air oven at 50°C, for 24 hrs. These feathers were pretreated in chloroform: methanol (3:1) solution for 48 hours and followed by drying at 40°C and it stored at 4°C.[11-13]

Isolation of Keratinase producing organism

Colony which grows well on feather supplemented pour plate was taken and streaked on Nutrient Agar plate. 5 different strains were taken and named as VITAMPS1,

VITAMPS2, VITAMPS3, VITAMPS4, and VITAMPS5 respectively.

Production of Keratinase enzyme

The isolated colonies obtained were inoculated in 50 ml of Nutrient Broth along with 1% (0.75 g) Emu feather as substrate and incubate at 37˚C in shaker with 120 rpm. Visual degradation of emu feathers was observed for 10 days. Keratinase assay can be performed as follows.[14,15] 20mg of sterilized feathers was taken and incubate with 0.2 ml of supernatant of five different strains in Tris HCL buffer with pH as 7 for 1 hour.[16-18] After incubation measure the absorbance at 280 nm. The OD values are taken for 10 days. The caseinolytic activity was performed using skim milk agar plate.[19,20]

Biochemical test

VITAMPS5 was grown on Nutrient Agar medium for fresh cultures. Spore production and localization were observed by microscopic observations.[21,22] The identification was done according to the technique described by Larpentand Larpent-Gourgaud (1985).[23,24]

Effect of pH and Temperature on Keratinolytic activity

Optimum pH of Keratinase enzyme can be determined using NaOH (20mM), Tris/HCl (2mM) in the different range from pH 1 to 11 at 37˚C. Optimum temperature for Keratinase enzyme was analyzed in different range from -4˚C to 90˚C. The Keratinase enzyme was measured as described previously.[25-27]

Effect of substrates on Keratinase production

Keratin rich material like hair, nail, emu feather, chicken feather, country hen feathers are used as substrates for Keratinase production. 1% different feathers were used in nutrient broth and inoculated with VITAMPS5. The culture condition was given as 37˚C at 120rpm and it was observed for 10 days.[28-30]

RESULTS AND DISCUSSION

Isolation of bacterial strains from soil sample

Soil sample were collected from different areas and different substrates. There are about 18 different colonies were found from emu feather dumped soil in superficial layer of soil and 20 strains were identified from same soil with 10 cm depth in Kilmonavoor, Vellore, Tamil Nadu. About 15 strains were found in hair dumped soil in the area of Abdhullapuram, Vellore, Tamilnadu and 18 colonies were found in the land of Sathupperi, Vellore, Tamil Nadu. About 20 colonies with different morphology were found in chicken feather dumped soil in Konavattam, Vellore, and Tamil Nadu. Of these colonies only five strains named as VITAMPS1, VITAMPS2, VITAMPS3, VITAMPS4, VITAMPS5 for identification were used to study, because these colonies degrade feathers well on the plate.

Caseinolytic activity of isolated bacterial strains

Keratinase is a proteolytic enzyme which hydrolysis protein and produces short peptides and amino acid as product. The protease enzyme is confirmed with milk agar plate assay. The culture free cells supernatant were collected and it is assayed on skim milk powder along with agar powder. Casein is the protein present in milk. It gives white cloudy appearance to white color. When casein undergoes hydrolysis it shows transparent in nature. In plate assay, skim milk contains casein and it is hydrolyzed by CFCS and it shows clear areas around the well. Of these five colonies VITAMPS1, VITAMPS3, VITAMPS4 and VITAMPS5 showed clear zone and it was taken for secondary confirmation for Keratinolytic activity.

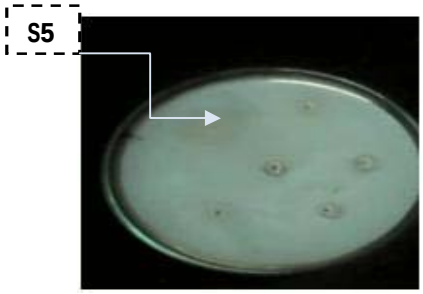


Figure 1: Caseinolytic activity of five different strains. 1-CFCS of VITAMPS5,2- CFCS of VITAMPS2, 3-CFCS of VITAMPS3, 4- CFCS of VITAMPS4, CFCS of VITAMPS1

Keratinolytic activity of five different strains

Feathers are used as carbon and nitrogen source for isolated bacteria to check keratinolytic activity. Nutrient broth were sterilized and feather was used as carbon and nitrogen source for bacteria which shows positive caseinolytic activity were selected and inoculated in broth. The control was served as NB supplemented with emu feather without inoculum. Emu feather consists of both white and black colour. The black colour feather are highy resistant because of presence of pigment. This pigment shows resistant to biological degradation of feathers. The strain VITAMPS5 showed degradation of feather after 7 days incubation at 37˚C and it confirmed the presence of keratinase enzyme.

Figure 2: Production media supplemented with feather after 8 days incubation with VITAMPS5

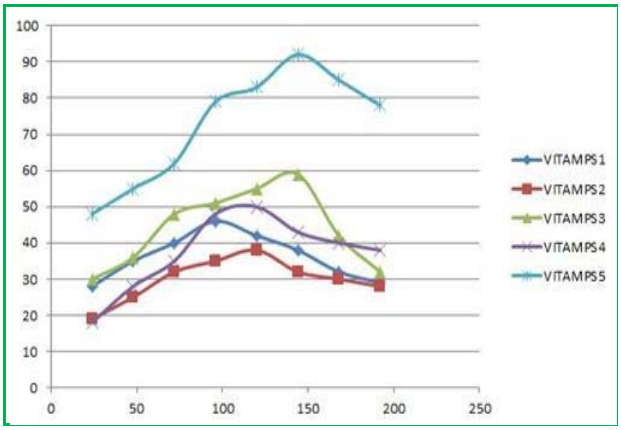
Microscopic view of feather degradation

The feathers in nutrient broth inoculated with VIAMPS5 were observed under bright field microscope in different days. From the figure, stage 1 showed control without any inoculation of VITAMPS5 in Nutrient broth .Stage 2

showed incubation of VITAMPS5 in Nutrient broth after 2 days and shafts in feathers were disturbed. In stage 3, VITAMPS5 was incubated after 4 days and 2 to 3 shafts were separated from center stick. In stage 3, VITAMPS5 was incubated after 6 days and center stick was affected. After 8 days incubation of VITAMPS5 in stage 5, small pieces of feathers was observed.

Table 1: Growth time for five different strains

Bacteria	Growth time (h)							
Keratinolytic Activity (U/ml)								
Days	24	48	72	96	120	144	168	192
VITAMPS1	28	35	40	46	42	38	32	29
VITAMPS2	19	25	32	35	38	32	30	28
VITAMPS3	30	36	48	51	55	59	42	32
VITAMPS4	18	28	35	48	50	43	40	38
VITAMPS5	48	55	62	79	83	92	85	78



X-axis: Number of days; Y-axis: Enz activity

Figure 3: Keratinolytic activity of five different strains

Biochemical Tests for characterization of VITAMPS5

Table 2: Biochemical characterization of VITAMPS5

Methods	Results
Gram staining	Positive and rod shaped cells
Spore staining	Positive with endospore
Oxidase test	Positive
Catalase test	Negative
Indole test	Negative
Methyl red test	Positive
VP test	Negative
Blood agar test	β-Hemolysis
Simion citrate test	Positive
TSI test	Acid with no gas formed

Effect of pH and Temperature

Maximum Keratinase activity was obtained from pH 9 to 11. This showed that Keratinase was alkaline character. The optimum activity in alkaline range showed wide range of biological applications in leather and textile industry. Optimum temperature was found to be 37˚C and relative activity was found to be at 60˚C.

Table 3: Effect of pH and Temperature on Keratinase

pH	Keratinolytic activity (U/ml)	Temperature (˚C)	Keratinolytic activity (U/ml)
1	37	0	20
3	40	4	28
5	50	25	35
7	88	37	89
9	119	60	82
11	150	80	54
13	84	95	28

Figure 4: Microscopic View of feathers. Stage 1- Control feather, Stage 2 - After 2 day's incubation, Stage 3 - After 4 day's incubation, Stage 4 - After 6 day's incubation, Stage 5 - After 8 day's incubation

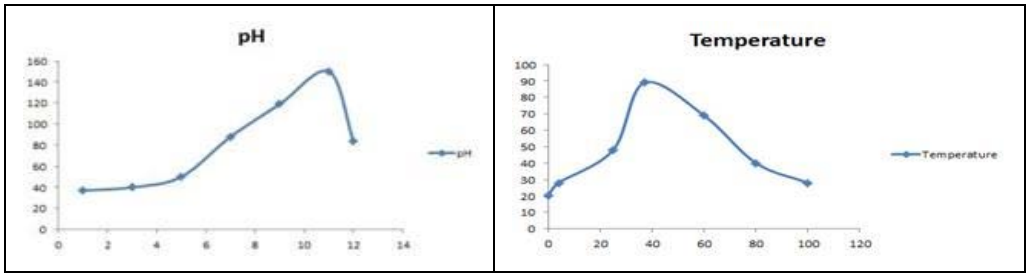


Figure 5: Effect of pH and Temperature (X-axis: pH, Temperature; Y-axis: Keratinolytic activity)

Effect of substrates on Keratinolytic activity

Most organisms produce inducible keratinase when substrates contain keratin rich material such as hair, nail, Chicken feathers, Country hen feathers. Feather was used as optimal source for keratinase production. Among all keratin rich material Chicken feathers were easily degraded by this strain followed by country hen. Both β and α keratin can be degraded by VITAMPS5. Thus VITAMPS5 had the ability to use different substrates and hence it is applicable to both keratin-degradation and keratinase enzyme production and hence amino acids of interest can be isolated.

CONCLUSION

Keratinase enzyme produced by VITAMPS5 showed maximum activity after 8 days incubation in Nutrient broth. The optimum pH and temperature for production of keratinase was found to be 11 and 37°C respectively. The strain VITAMPS5 was found to be gram positive, motile and aerobic hence it may be conclude that this strain may be Bacillus species from biochemical tests. The alkaline nature if keratinase showed that it had wide application in leather industry, detergent industry and helps in hydrolysis of prions, nutritional feed for animals and source for amino acids. Thus emu feathers are used as source for production of aminoacids.

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