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Veenayohini K
Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamilnadu, India

Sangeetha D
Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamilnadu, India

Correspondence
Veenayohini K
Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamilnadu, India

Isolation and identification of keratinolytic bacteria from poultry waste and assessment of its keratinase activity on chicken feathers

Veenayohini K and Sangeetha D

Abstract
Keratinase producing bacterial strains were isolated from soil and feather samples from chicken feather dump site and identified by physical and biochemical methods. Keratinase producing potential of keratinolytic strains (*Bacillus subtilis* and *Bacillus licheniformis*) were checked at various physical and chemical parameters. The maximum Enzyme activity was showed by *Bacillus subtilis* then compared with *Bacillus licheniformis* in all the optimization studies include, pH (32.2 ± 1.1 at pH 8), Temperature (36.2 ± 0.41 at 70 °C), Substrate concentration (29.0 ± 0.60 at 0.6 g), metal ions (27.2 ± 0.04 at Cd2+). The raw feather degradation studies shows, the *Bacillus subtilis* have great feather degradation potential (79%) then *Bacillus licheniformis*. The *Bacillus subtilis* colonization on treated feathers was examined under scanning electron microscopy.

Keywords: Keratinase, enzyme activity, feather and *Bacillus*

Introduction
Keratin is an insoluble protein molecule having very high stability and low degradation rate. It is majorly present in feather, hair, nails, wool and horns (hard keratins) and stratum corneum (soft keratins) of animals and birds. These proteins which belong to the scleroprotein groups are tremendously resistant to the action of chemical, physical and biological degrading agents (Karthikeyan *et al.,* 2007) [9]. Important amino acid presented in keratin is Cysteine, Arginine, Serine, and Glycine.

The enzyme Keratinases have an ecological importance and protects the environment from feather wastes pollution and play a vital role in degradation of such wastes in a place like Keoladeo National Park, Bharatpur, India. Such materials can provide a propagation spot for various types of pathogenic microorganisms. Several studies were focused on production, purification, molecular weight and keratinolytic ability of keratinases towards different keratinaceous substrates. The purified keratinase from feather degrading bacterium was extensively used to partial hydrolyses of feathers and converted them into a digestible feed protein (Shih *et al.,* 2006) [22]. The use of these enzyme as an alternative to dehairing catalyze in leather industry in slow release nitrogen fertilizer, cosmetics and bio-degradation films (Riffel *et al*., 2003a) [19].

Keratinolytic microorganisms and their enzymes may be used to enhance the digestibility of feather keratin. They may have important applications in processing keratin-containing wastes from poultry and leather industries through the development of non-polluting methods (Onifade *et al*., 1998) [18]. A number of keratinolytic microorganisms have been reported, including some species of *Bacillus* (Atalo and Gashe, 1993) [1], actinomycetes (Bockle *et al.,* 1995) [2] and fungi (Kushwaha, 1983) [12]. The mechanical stability and high resistance to proteolytic degradation shown by this protein (keratin) are due to their disulphide bonds, hydrogen bonds, salt linkage and cross linking. Increasing attention is focused on keratinolytic microorganisms and microbial keratinases due to their potential application in the bioconversion of keratin-rich wastes generated from meat (particularly poultry) and leather industries, through the development of ecologically safe and economically feasible processes. Additionally, keratinases are postulated for utilization in food, feed, detergent, leather, and biomedical industries (Brandelli *et al*., 2010) [3].

Nowadays feather waste is utilized as a dietary supplement for animal feed stuffs. Physical and chemical treatment for conversion of feathers into food supplement can destroy amino acids and decrease protein quality. Use of microbial Keratinase for keratin degradation is the innovative solution for recycling feather waste and reducing pollution Conversion of feathers into feather meal, dietary protinfor animal feed by using physical and chemical treatment is significant. These methods can destroy certain amino acids and decrease protein quality and digestibility. Physical and chemical methods can lead to destruction of amino acids as well as decrease the protein content and digestibility. The utilization of agro-industrial residues may increase energy conservation and recycling. To overcome the loss of aminoacids due to keratin hydrolysis microbial keratinases are used. Keratin materials in the form of agro-industrial waste increasingly accumulate in the environment. The extremely resilient nature of this protein gradually leads to problematic ecological issues. Growing demand for efficient alternative to traditional recycling techniques guides towards application of keratinolytic microorganisms in the bioconversion process. Among numerous microbial groups many keratin degraders derive from the bacterial genus *Bacillus*. The present study was under taken with the following objectives of isolation and identification of keratinolytic microorganism from soil sample collected from poultry waste dumping yard and assessment of keratinase activity.

Materials and Methods

Sample collection and Serial dilution

The soil and feather samples were aseptically collected in sterile air seal polyethylene bags from the poultry farm in Chidambaram, Tamil Nadu. The collected samples were immediately transferred into lab and stored in refrigerator condition. Serial dilution for each sample was prepared by adding 1 g of the soil sample to 9 ml of sterile saline. Then serial dilution up to 10^{-8} was done using sterile saline and from appropriate dilutions (10^{-7} and 10^{-8}) 1 ml of sample was poured into the Nutrient Agar plates respectively. Three replicates and control plates was maintained for the calculation of Colony Forming Unit (CFU). All inoculated and control plates were incubated at 37 °C for 24 hours.

After the incubation, nine different bacterial colonies were picked up by inoculation loop and it's were streaked on respective plates containing nutrient agar. Then the colonies were continuously streaked on nutrient agar palates by different streaking plate methods until get purified colonies of isolates. Isolated nine different purified colonies were sub cultured on nutrient agar slants.

Screening for Keratinolytic activity

Preparation of inoculums and feather meal

One loop full of nine different bacterial isolates was taken from nutrient agar slants and inoculated into the conical flasks containing sterilized nutrient broth. Inoculated broths containing conical flasks were incubated at 37 °C for 24 hours and it was used as inoculums for screening of keratinase activity of isolates. Poultry feathers was washed extensively, boiled at 30-40 psi for 2-3hrs and dried in hot air oven at 50 °C for 4 hours. The dried feathers were pulverized and the powder was used as feather meal.

The plates containing feather meal agar plates were prepared and wells were formed on agar plates by removing agar using 6 mm diameter cork borer. The wells were filled with 0.1 ml of isolated bacterial cultures broth respectively and the plates were incubated at 37 °C for 24. Around the well, clear zone forming three isolates were selected for the further work.

The strain was further grown on nutrient broth containing feather meal and kept for incubation at 40 °C for 7days with shaking at 150 rpm. Then the culture supernatant was assayed for keratinolytic activity. The strain, which degraded keratin effectively and it was further identified by physical and biochemical tests. Totally two isolates shows the keratinolytic activity on feather meal agar plates, that two isolates were named as KB1 and KB2 and then taken for growth and identification study. The keratinolytic isolates were partially identified using various staining procedures and bio- chemical tests prescribed by Bergey's manual of systematic bacteriology IV Edition [Vos, 2009].

Growth measurements of Keratinolytic bacteria

The isolated bacterial strains KB1and KB2 were studied for their potential growth rate in minimal feather meal broth. One loop full of bacterial inoculums was transferred into the flash containing feather minimal media and the flasks were incubated at 37 °C for 7 days. The growth rate was observed by taking OD values in UV visible spectrophotometer at600 nm for every 12 hours interval.

Enzyme assay

Crude enzyme preparation

The broth was incubated with isolated Keratinolytic bacteria at 40 °C for 7 days with shaker at 150 rpm and it was centrifuged at 10,000 rpm for 5 min. the supernatant was transferred into another clean test tube and it was used as crude enzyme.

Preparation of keratin solution

Keratinolytic activity was measured with soluble keratin (0.5%, w/v) as substrate. Soluble keratin was prepared from white chicken feathers by the method of (Wawrzkiewicz *et al.,* 1987) [25]. Native chicken feathers (10 g) in 500 ml of dimethyl sulfoxide were heated in a hot air oven at 100 °C for 2 h. Soluble keratin was then precipitated by addition of cold acetone (1 L) at −70 °C for 2 h, followed by centrifugation at 10,000 rpm for 10 min. The resulting precipitate was washed twice with distilled water and dried at 40 °C in a vacuum dryer. One gram of quantified precipitate was dissolved in 20 ml of 0.05M NaOH. The pH was adjusted to 7.0 with 0.1M Hydrochloric acid and the solution was diluted to 200 ml with 0.05 mol/L Phosphate buffer (pH 7.0).

Keratinase assay

The keratinolytic activity was assayed 1.0 ml of crude enzyme properly diluted in Phosphate buffer (0.05 M of pH 7.0) was incubated with 1 ml keratin solution at 50 °C in a water bath for 10 min, and the reaction was stopped by adding 2.0 ml 0.4M Trichloro acetic acid (TCA). After centrifugation at 1450xg for 30 min, the absorbance of the supernatant was determined at 280 nm against a control. The control was prepared by incubating the enzyme solution with 2.0 ml TCA without the addition of keratin solution. One unit (U/ml) of keratinolytic activity was defined as an increase of corrected absorbance of 280 nm (*A* 280) (Gradisar *et al*., 2005) [8] with the control for 0.01 per minute under the conditions described above.

Estimation of enzyme activity

Effect of pH

The optimum pH of the crude enzyme was found by dissolving the keratin at various buffers using 0.05 mol/L acetate buffer (pH 4-5.5), 0.05 mol/L phosphate buffer (pH 6-7.5), 0.05 mol/L Tris-HCl (pH 8-9), Based on the above mentioned pH the keratin solution is prepared for various buffers. The experiment on the effect of pH on enzyme stability was carried out by incubating the enzyme solution at pH ranges of 4-9. Then the enzyme activity was determined by the standard enzyme assay.

Effect of temperature

With the optimum pH of the crude enzyme as constant the optimum temperature was found by incubating the enzyme with the substrate (keratin solution prepared according to optimum pH) at varying temperature range from 30 to 80 °C. The experiment on the effect of temperature on enzyme stability was carried out by incubating the enzyme solution at temperature ranges of 30-80 °C Then the enzyme activity was determined by the standard keratinase enzyme assay.

Effect of Substrate concentration

The km and Vmax value for the crude keratinase is determined by using different concentrations of keratin. For this stock solution of keratin is prepared by dissolving 0.5 g in 50 ml phosphate buffer of pH 7. The stock solution is diluted for different concentrations (0.1-0.6 g) in a series of test tubes using phosphate buffer.

Effect of Metal ions

To study the influence of enzyme inhibitor, crude keratinase (1ml) was incubated with varying concentration (1-3mM) of 1.10 Phenanthroline and EDTA in 0.05mol/l phosphate buffer (pH 7) at 37 °C for 30min. The effect of divalent cation on keratinolytic activity was determined by incubating the crude enzyme in the presence of Zinc, magnesium, copper, mercury, cadmium (Zn2+, Mg2+, Cu2+, Hg2+, Cd2+) at (1-3mM) concentration for 1h at 37 °C. Then the enzyme activity was determined using lysis of feather powder as described above

Degradation of raw feather by Keratinolytic bacteria

Keratinolytic organisms were cultured in nutrient broth for 7-8 h. Then it was transferred to flask containing liquid basal medium (Riffel *et al.,* 2003) [20] where whole feather was fragmented into pieces with about 1 g and added to the basal media as a sole source of carbon and nitrogen. The flask was incubates 47 °C for 7 days with 120 rpm shaking. The percent of keratinous waste degradation was determined.

Determination of degree of degradation (DD)

The residual feather was washed, dried and scaled to calculate DD by using following equation (Kim *et al*., 2011).

DD (%) = (TF-RF) ×100/TF

Where, TF is the total feather and RF is the residual feather

Scanning electron microscope for surface modification on raw feather

The degradation of the feathers was characterized by SEM. The culture broth containing degraded feathers were filtered, washed twice by distilled water and the substrates were dried. The degraded feather and the non-degraded feather were pasted separately in a stub using double sided adhesive carbon conducting tape and gold sputtered. The sample was analyzed in the scanning electron microscope (Hitachi S-3000 H) using photomultiplier detector and images were taken at 50 X and 250 X.

Experimental Results

In the present study, the keratinase enzyme producing bacteria was isolated from feather and soil samples by serial dilution technique collected from poultry farm in Chidambaram, Tamil Nadu. Further the isolates were cultured screened for enzyme activity it was used to raw feather degradation.

Isolation and enumeration of total bacterial load in collected sample

The population of bacteria in the samples was enumerated. The poultry soil sample shows maximum number of colonies on nutrient plate with 132 ± 2.4 CFU/g at 10^{-7} dilution factor followed by the 10^{-8} dilution factor with 71 ± 0.9 CFU/g. in poultry feather sample the maximum bacterial load was enumerated in 10^{-7} dilution factor 114 ± 3.3 CFU/g followed by 10-8 with 52 ± 3 CFU/g. this results were showed in Table-1. Based on colony morphology, nine different bacterial colonies were purified by streaking methods.

Table 1: Estimation of total colony forming units in sample

Sample	Total colony forming unit (CFU/g)	
	Dilution factor	
	10^{-7}	10^{-8}
feather	114 ± 3.3	52 ± 3
Soil	132 ± 2.4	71 ± 0.9

Screening for keratinase production

The purified nine isolates were named as isolate- 1, isolate-2,……. Isolate- 9. All the isolates were screened for keratinase enzyme production by agar well plating method on feather meal agar plates. Among the nine bacterial isolates, two isolates namely isolate- 2 (14 mm) and isolate- 6 (17 mm) showed the clear zone around the well after the incubation. Other seven isolates did not produce zone around the well. That two isolates were taken for further study and again the isolate-2 and isolate- 6 were named as KB1 and KB2. The results were showed in Table- 2.

Table 2: screening for keratinolytic activity

Isolates	Isolate-1	Isolate-2	Isolate-3	Isolate-4	Isolate-5	Isolate-6	Isolate-7	Isolate-8	Isolate-9
Zone around the well (mm)	---	14	---	---	---	17	---	---	---

(---); No enzyme activity

Growth study of Keratinolytic bacteria

Growth rate of bacterial isolates KB1 and KB2 was assessed in UV visible spectrophotometer at 600 nm for every 12 hours interval. The KB1 starts their lag phase after inoculation and it shows its lag phase up to 24 hours. Log phase of KB1 was started after 24 hours and it was continued up to 160th hour after that stationary phase was continued up to 132nd hour. KB2 was starts its log phase after inoculation and it was continued up to 36th hour. After 36th hour the KB2 shows its stationary phase and it was continued up to 144th hour and

after its death phase was started. The high OD value of 51was observed at 132 hours incubation and by KB1 and least value was observed in KB2 the OD value of 0.13. The results were showed in Figure- 1. Both the keratinolytic strains have long time growth rate in the liquid medium.

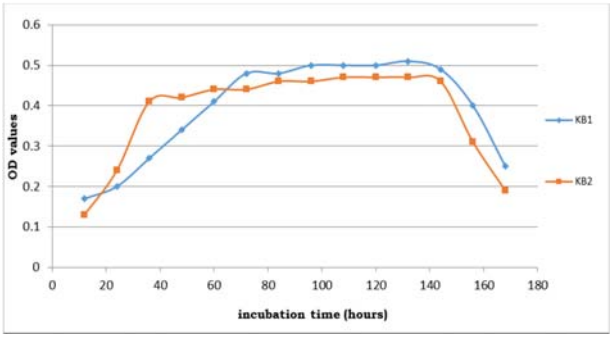


Fig 1: Growth rate of bacterial keratinolytic strains

Identification of keratinolytic bacterial isolates

After the biochemical and morphological characteristics assessment the results were compared with bergey's manual of systematic bacteriology *IV* edition. Based on biochemical, morphological and microscope characteristics the isolates of KB1 and KB2 is Gram positive, Rods in shape , arranged in short chains and motile. The strain KB1gives positive results in Starch reduction, Indole, MR, Citrate, Starch reduction tests and gives negative results in Catalase, Oxidase, Gelatinase, VP tests. The strain KB2 gives positive results in MR, VP, Catalase, Oxidase tests and gives negative result in indole and gas production tests. According to the results obtained from the biochemical test partially the isolates were conformed as *Bacillus subtilis* (KB1) and *Bacillus licheniformis* (KB2).

Estimation of keratinase enzyme activity

Effect of pH

The effect of pH on enzyme production is depicted in Figure-2. The results indicates both the keratinolytic bacterial strains *Bacillus subtilis* and *Bacillus licheniformis* is an alkalophilic bacterium with a broad range of pH (6, 7, 8 and 9) for enzyme activity. *Bacillus subtilis* shows the maximum enzyme activity at the pH of 8 with the enzyme activity of 32.2 ± 1.1 U/ml and the keratinolytic strain *Bacillus licheniformis* shows the highest enzyme activity of 23.1 ± 0.10 U/ml at pH 7 and 8 followed by pH 9 with the enzyme activity of 19.1± 0.21 U/ml. In acidic pH (4 and 5) both the bacterial strains shows least enzyme activity. The lowest enzyme activity was recorded in pH 3.2 ± 0.8 U/ml by *Bacillus licheniformis.*

Effect of temperature

The effect of temperature on enzyme activity is depicted in Figure- 3. The results indicate both keratinolytic bacterial strains showed the enzyme activity on wide range of temperature (40, 50, 60, 70 and 80). *Bacillus subtilis* shows highest enzyme activity at 70 °C with enzyme activity of 36.2 ± 0.41U/ml followed by the enzyme activity of 31.3 ± 0.12 U/ml at 60 °C. The *Bacillus licheniformis* shoes the maximum enzyme activity of 31.1 ± 0.10 U/ml at 70 °C followed by the enzyme activity of 30.1± 0.21 U/ml at 70 °C. The least amount of enzyme activity was produced by *Bacillus licheniformis* (3.2 ± 0.8 U/ml) at 30 °C. Both the bacterial strains effectively produce the enzyme and denature the keratin at thermophilic temperature that indicates the both strain are thermophilic in nature.

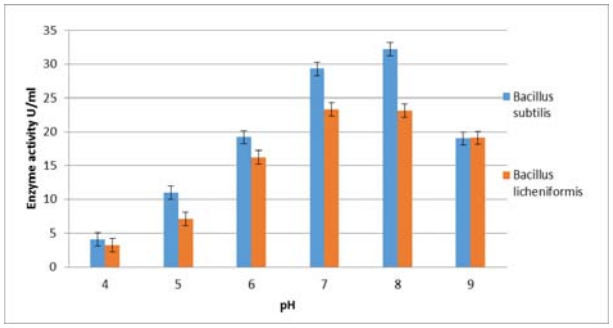


Fig 2: Effect of pH on enzyme activity

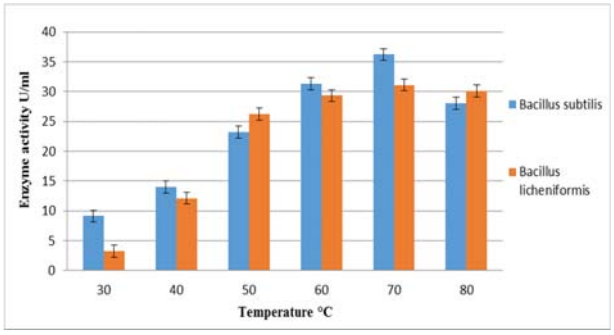


Fig 3: Effect of temperature on enzyme activity

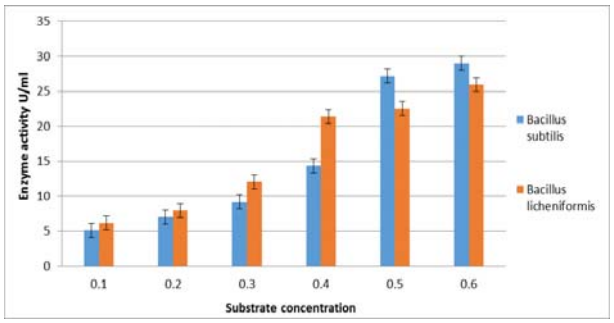


Fig 4: Effect of substrate concentration on enzyme activity

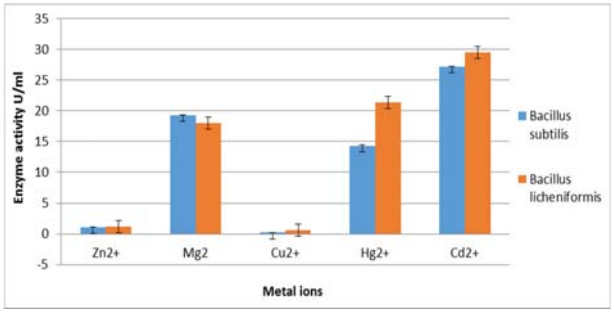


Fig 5: Effect of metal ions on enzyme activity

Effect of substrate concentration

The effect of substrate concentration of soluble keratin on enzyme activity was assessed and the results were showed in Figure- 4. Both the keratinolytic strains showed the increasing enzyme activity at while the increase of substrate concentration in the reaction mixture. The *Bacillus subtilis* and *Bacillus licheniformis* showed the maximum enzyme activity at the substrate concentration of 0.6 with the enzyme

activity of 29.0 ± 0.60 U/ml and 26.0± 0.24 U/ml. The least value of enzyme activity was recorded at substrate concentration of 0.1 with enzyme activity of 5.1 ± 0.14 U/ml by *Bacillus subtilis*. According to these results the bacterial strains shows the enzyme activity in increasing in nature while the substrate concentration was increased.

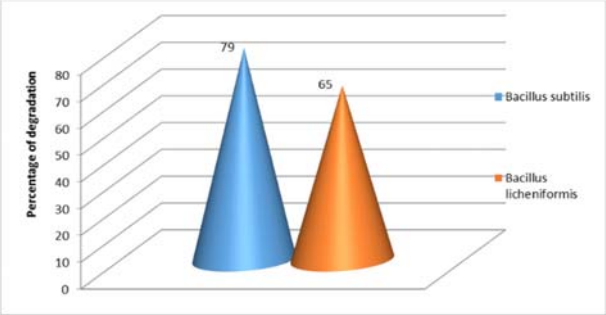


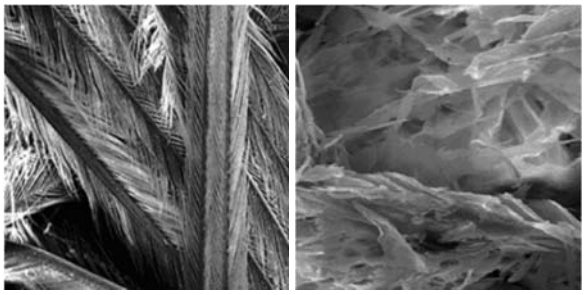
Fig 6: Degree of degradation of raw chicken feather

Effect of metal ions

The effect of metal ions on enzyme activity was assessed and the results were showed in Figure- 5. The results indicate both the keratinolytic bacterial enzyme activity was highly suppressed by the $Zn2^{+}$ ions followed by $Cu2^{+}$ ions. The maximum enzyme activity by *Bacillus subtilis* was resulted in $Cd2^{+}$ ions involved enzyme reaction with enzyme activity of 27.2 ± 0.04 U/ml followed by the enzyme activity of 19.3 ± 0.10 U/ml at $Mg2^{+}$. *Bacillus licheniformis* shows maximum enzyme activity of 29.5 ± 0.01 U/ml by the involvement of $Cd2^{+}$ in enzyme reaction. The least enzyme activity was recorded by $Cu2^{+}$ with the enzyme activity of 0.2 ± 0.01 U/ml.

Degradation of raw chicken feather

The degradation of raw chicken feather by isolated keratinolytic bacterial strains was assessed and the results were showed in Figure- 6. The maximum percentage of degree of degradation of chicken feather was observed in the feather inoculated with *Bacillus subtilis* with degree of degradation of 79% followed by *Bacillus licheniformis* with the degree of degradation of 65%. According to these results the both the bacterial strains degrade the chicken feathers in liquid medium.



a) b)

Fig 7: Scanning electron microscopic images of treated chicken feathers at 50 X (a) and 500 X (b) magnification.

Scanning electron microscopic analysis

The treated feather inoculated with *Bacillus subtilis* was subjected to the Scanning electron microscopic analysis at 50 X and 250 X magnification for surface modification and bacterial colonization on feather. Scanning electron microscopic images indicates the feather surface was highly ruptured by *Bacillus subtilis.* The colonization of *Bacillus subtilis* on chicken feather also analyzed. The images indicate the mass colonies were found in the surface of the feather. The Scanning electron microscopic images were showed in Figure- 7.

Discussion

The bacterial population in collected soil and feather samples were analyzed and the total colony forming unit was calculated. The results indicates the both the soil and feather samples were act as a natural habitat for wide verity of bacterial species. Microbial load in the feather is act as the reservoir for the keratinolytic microbes of bacteria, fungi and actinomycetes species.

The results obtained from this present study, keratinolytic bacteria was presented in chicken feather and feather dumping sire soil along with different verity of non keratinolytic bacterial species. The isolated keratinolytic bacterial species were partially identified as *Bacillus subtilis* and *Bacillus licheniformis*.

Similarly Lin *et al*. (1995) [13] reported that keratinolytic bacterial strains were isolated from soil and other natural sources and that was identified as *Bacillus sp.* as a potential keratinolytic organism and its possible use in field studies for biodegradation of feather was studied. The bacterial keratinolytic strain of *Bacillus altitudinis* was isolated in previous study conducted by Vijayakumar *el al.* (2011) [24].

Kulkarni and Jadhav, (2014) [11] for isolation of potent strain of keratinolytic microbe, all strains were inoculated in modified basal salt media along with feathers and incubated at 140 rpm for 3-5 days in shaker incubator. Visual detection of feather degradation was carried out every after 24 hrs. It was found that N7 (2) strain degrade feathers within 72 hrs, and having keratinolytic potential. All these strains were also grown on Hicrome *bacillus* agar for rapid identification of the isolates.

Whereas previously described keratinolytic bacteria mostly have keratin degrading potential at ambient temperatures (Mohamedin, 1999) [16]. However, these strains perform similar to a *Vibrio* strain kr2, previously isolated from decomposing feathers (Sangali and Brandelli, 2000) [21]. An optimum keratin degrading activity at mesophilic temperatures should be a attractive characteristic because these microorganisms may achieve hydrolysis with reduced energy input.

Previous investigations have described the effect of cultivation conditions aiming to optimize the production of bacterial proteases, and these different circumstances may be subjected to comparisons. Keratinase enzyme production by *Chryseobacterium* sp. kr6 was significantly affected by initial pH and temperature, with maximum yield at 23 °C, initial pH 8.0–9.0, and raw feathers at 30 g L^{-1} (Casarin *et al*., 2008) [4]. For *Serratia rubidaea*, maximum protease production was achieved after 48 h in a medium containing starch (8 g L^{-1}), salt (6.25 g L^{-1}), casein (4 g L^{-1}) and initial pH 8.0 (Doddapaneni *et al.,* 2007) [6]. Temperature of cultivation also considerably affected the production of keratinase by *Microbacterium* sp and optimum conditions were 25 °C, initial pH 7.0, and 12.5 g L^{-1} of feather meal (Thys *et al.,* 2006).

Feather meal (substrate) concentration, initial pH and temperature initial pH were also important factors on

keratinase production by *Streptomyces* sp7, which was maximal at 7 g L^{-1}, 45 °C, and pH is 11.0, respectively. However, keratinase production might also be achieved without a keratinous substrate on culture media. For instance, keratinase production by *Bacillus licheniformis* ER-15 was maximal at 37 °C with glucose as carbon source and soy flour as nitrogen source (Tiwary *et al*., 2010) [23].

Through the strategy of isolation of keratinolytic bacteria utilized in this work, *Bacillus* spp. presenting high keratinolytic activity was selected. Considering that feather meal has been showed to be an potential source of metabolizable protein (Klemersrud, 1998) [10], and that bacterial keratinases increase the digestibility of feather keratin (Odetallah *et al*., 2003) [17], these keratinolytic bacterial strains could be used to produce animal feed protein. In addition, the selected isolates were able to grow and display keratinolytic activity in diverse keratin wastes. This would be beneficial for the utilization of these residues.

The study conducted by Manju, (2012) [15] *Bacillus* sp was achieved in 96 hours of incubation at 30 °C in trace salts medium. 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 great potential for the 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.

The enzyme activity was studied for *Bacillus subtilis* and *Bacillus licheniformis* over the broad range of temperature (30- 80 °C) and it is found to be optimal at 50 °C. Further increase the temperature to 80 °C reduces the relative activity. The high thermo stability allows performance of industrial bio-conversion process at high temperature to minimize the risks of microbial contamination. Keratinolytic bacteria often exhibit optimal growth and activity at higher temperature (Lin *et al*., 1992) [14]. However the induction of keratinolytic enzyme produced by the species of *Bacillus* with feather powder, human hair and nails, guinea pig hair and cow horn and hooves was reported (Cheng *et al*., 1995) [5]. An increase in pH values was observed during feather degradation, a trend similar to other microorganisms with large keratinolytic activities (Sangali and Brandelli, 2000) [21]. This trend may be associated with proteolytic activity, consequent de-amination reactions and the release of excess nitrogen as ammonium ions. The increase in pH during cultivation is piercing as an important sign of the keratinolytic potential of microorganisms.

Conclusion

According to the results obtained from this present study, the strains *Bacillus subtilis* and *Bacillus licheniformis* are widely associated with chicken feathers and soil of feather dumping site. Both the isolates effectively degrade the keratin substrates at wide range of pH and temperature. This character of both bacterial isolates is leads to wide range of keratinase activity, among these characteristics of isolates is well adaptive for the large scale bio- conversion of keratin waste. The both bacterial strains show potential keratinase activity on raw chicken feathers. The industrial application of these strains for large scale bio conversion of keratin waste into poultry feed stuff creates significant importance.

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