

Isolation, characterization and optimization of keratinolytic bacteria from chicken feather waste dumping site

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ABSTRACT

Chicken feathers are considered to be the waste products of poultry industry and can create a solid waste problem in the environment. Keratinases have the potential to degrade insoluble keratin predominantly present in feathers, hair, horns, and hooves. The aim of present study is to isolate, screen, and identify keratinolytic bacteria from feather waste dumping site and to optimize the culture conditions for maximum keratinase production and subsequent degradation of feathers. Fourteen bacteria were isolated from the feather waste dumping site at Virudhunagar, Tamil Nadu, India, and were screened for their keratinolytic property. Comparatively, three bacteria displayed better keratinolytic activity. Based on morphological and biochemical characteristics and 16S rRNA gene sequence analysis, they were identified as *Bacillus licheniformis*, *Glutamicibacter arilaitensis*, and *Serratia marcescens*, respectively. The effect of temperature, pH, feather concentration, and various substrates on the growth parameters of those bacteria was studied. The growth and protein production was high at 40°C for all bacteria. *B. licheniformis* and *S. marcescens* produced more protein at pH 8.5, whereas *G. arilaitensis* produced more protein and grown well at pH 8. Hence, three bacteria were subjected to submerged fermentation using chicken feather powder (1%) as Carbon and Nitrogen sources. Among them, *G. arilaitensis* is the superior in degrading feathers producing more protein (2.15 ± 0.04 mg/ml) and amino acids (0.498 ± 0.019 µg/ml). The microscopically observed smears of feather hydrolysates revealed that *G. arilaitensis* degraded the chicken feathers more efficient comparatively other two bacteria.

1. INTRODUCTION

The poultry industries largely satisfy the ever increasing animal protein demand globally that has arisen due to population explosion [1]. People consume large quantity of chicken meat, consequently leads to the enormous generation of slaughterhouse waste [2]. Globally, 8.5 billion tons of poultry feather waste has been produced annually. In 2018, India produced 4.6 million tons of broiler chicken [3] and approximately 350 million tons of feather waste generated every year [4] and the feathers are indiscriminately dumped into the environment [5]. The accumulation of feathers in the environment leads to a variety of human ailments including chlorosis, fowl cholera, mycoplasmosis, and the outbreaks of H5N1 virus [6,7]. More than 90% of feathers are composed of protein and the major one is keratin. Extensive disulfide bonds and cross-linkages make keratin rigid and insoluble in water. Based on the sulfur content, keratins are classified as: hard keratins (feather, hair, hoof, and nail) and soft keratins (skin and callus) [8]. Sulfur content in keratin is high due to the presence of sulfur containing amino acids such as cystine, cysteine, and methionine [9,10]. Keratin is remarkably resistant to degradation

by regular proteolytic enzymes such as pepsin, papain, and trypsin. Nevertheless, feathers are considered as a good source of protein and essential amino acids if systematically recycled [11].

Various strategies have been adapted to manage the slaughterhouse waste generated in India. However, feather waste still remains a challenge [12,13].

Conventional methods of feather degradation such as acid/alkali hydrolysis and steam pressure cooking destroy the heat sensitive essential amino acids such as lysine, methionine, and tryptophan and generate non-nutritive amino acids apart from consuming huge amount of energy [14-16]. Land dumping and incineration methods are likely to result in environmental destruction and generates highly toxic emissions [17]. The conventional method of processing feather waste is cost and labor intensive that may reduce the nutritive value. Protease is used for hydrolysis of the peptide bonds that link amino acids together in a polypeptide chain [18,19]. The enzyme-catalyzed reaction is cost effective, highly efficient, and easily selective which usually requires mild reaction conditions and less energy which consequently improves the value of feathers [20]. A group of secreted proteolytic enzymes called as keratinases have the ability to hydrolyze insoluble keratins more efficiently than other proteases, are produced by microorganisms and some insects [21]. Hence, the alternative method of using microorganisms and their enzymes in

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feather processing technology is an environmental friendly approach. A myriad of actinomycetes, bacteria, fungi, and algae are known to produce keratinases to degrade keratin [22,23] and degradation of poultry feathers by microbial keratinases might afford a feasible alternative technique for bioconversion of keratin.

Keratinase is an extracellular enzyme produced in the presence of keratin at a broad range of pH and temperature which break the disulfide bond in keratin during degradation process. Soil holds a large number of phylogenetically diverse keratinophilic and keratinolytic microorganisms which produce enzymes with good stability at higher temperatures and alkaline conditions [24]. Microorganisms such as bacteria (*Bacillus pumilus*, *Micrococcus* sp., *Microbacterium* sp., and *Pseudomonas* sp.), actinomycetes (*Streptomyces* sp. and *Thermoactinomyces candidus*), and several fungi (*Alternaria radicina*, *Aspergillus* sp., *Doratomyces microspores*, and *Rhizomucor* sp.) have been reported to produce keratinase [25]. Recently, the production of keratinases using heterologous expression systems such as *Escherichia coli*, *Bacillus* sp. and *Pichia pastoris* in insect cells has attracted significant research interest [26]. Microbial keratinases are generally extracellular enzymes that are inducible in nature and some are cell bound and intracellular. Keratinases are classified into exoprotease and endoprotease based on their cleavage site as well as have many applications in leather, detergent, cosmetics, and pharmaceutical industries [27]. Hence, microbial keratinases have potential to conquer the distinctive position among the proteases which have an impact on environment to bioprocess keratinous waste. Keratinolytic bacteria play an important role in the conversion of chicken feather keratin into amino acids and soluble protein. Consequently, there is a need for new process enabling ecological and economical valorization of this resistant chicken feather waste. Thus, the present study is to isolate and characterize keratinolytic bacteria and optimize their culture conditions for better degradation of chicken feather.

2. MATERIALS AND METHODS

2.1. Preparation of Chicken Feather Powder

Feather powder was prepared by the method of Larasati *et al.* [28] Chicken feathers were collected from a slaughterhouse at Virudhunagar, Tamil Nadu, India. The collected feathers were washed with detergent followed by water until the blood stains and dirt were removed and feathers were dried in sunlight. Then, the feathers were cut into small pieces and stored in an airtight plastic container.

2.2. Isolation and Identification of Keratinolytic Bacteria

The soil samples were collected from feather dumping site at Virudhunagar (9°35' 13.9524" N Latitude and 77°57' 5.1516" E Longitude), Tamil Nadu, India. Soil samples were collected from 3 to 4 cm depth using a sterile spatula and transferred to sterile zipper polythene bags and brought to the laboratory, Department of Zoology, Thiagarajar College, Madurai. The serial dilution was performed by following standard procedure [29] and diluted soil samples (10^{-3} , 10^{-5} , 10^{-7} , and 10^{-9}) were inoculated on feather meal agar plates (the media composition consists of (g/l): 0.5 NH_4Cl ; 0.5 NaCl ; 0.3 K_2HPO_4 ; 0.4 KH_2PO_4 ; 0.1 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 10 feather powder; and 15 agar) and incubated at 37°C for 72 h. The well grown keratinolytic bacteria in the feather meal agar were isolated and maintained on agar slants. Further, all the bacteria that exerted better activities were identified and have taken for further studies. The isolated microorganisms were identified by morphological and physiological characteristics and they

were acknowledged based on the prescriptions of Bergey's Manual of Systematic Bacteriology [30].

2.3. Molecular Identification of Bacteria

2.3.1. Bacterial genomic DNA isolation

The isolation of bacterial genomic DNA was carried out following Sankari and Khusro [31] method. Two milliliters broth culture of bacteria was centrifuged at 7000 rpm for 5 min and the supernatant was discarded. One milliliter UniFlex™ buffer 1 and 10 μl RNase were added and mixed well to the obtained pellet by pipetting and incubated for 30 min at 37°C in a water bath. Then, 1 ml phenol-chloroform mixture (1:1) was added and mixed well. Then, the sample was centrifuged for 15 min at 10,000 rpm a room temperature and separated the aqueous layer in a fresh vial (1.5 ml). Again 1 ml UniFlex™ buffer 2 was added to the aqueous layer and mixed well by pipetting. The mixture was centrifuged at 12,000 rpm for 15 min at room temperature, the supernatant was discarded and 500 μl ethanol (70%) was added to the pellet and centrifuged again at 10,000 rpm for 10 min at 4°C. Finally, the obtained pellet was air-dried until it completes the evaporation of ethanol. The pellet was re-suspended in 50–100 μl UniFlex™ elution buffer and the obtained DNA was stored at -20°C.

2.3.2. Amplification of 16S rRNA genes

The amplification of 16S rRNA gene fragment was carried out using universal primers, forward (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse (5'-GGTACCTTGTTACGACT-3') [32]. The PCR cycling conditions were as followed: denaturation for 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. The DNA amplification reaction mixture (30 μl) consisted of 2X Amplicon Red master mixes (amplicon®), with genomic DNA (10 ng total) and 10 pmol of forward and reverse primers were used. After amplification, the PCR products were electrophoresed in 1% agarose gel and stained with ethidium bromide. The stained gel was photographed using a gel documentation system. The amplified fragments were purified and sequenced by Eurofins Scientific (Bangalore). The sequences were compared with the GenBank database using National Center for Biotechnology Information (NCBI) BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for species level identification and phylogenetic trees were constructed using the neighbor-joining algorithm in Molecular Evolution Genetic Analysis version 8.0.

2.4. Effect of Different pH on Bacterial Growth

The effect of different pH on the growth of bacteria was studied. Overnight broth culture was inoculated into the feather minimal medium prepared at different pH values such as 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10, and 10.5 and incubated in shaking incubator for 120 h. The bacterial growth was estimated at every 24 h interval by reading the absorbance at 620 nm against a control blank without inoculums.

2.5. Effect of Different Temperature on Bacterial Growth

The impact of diverse temperatures on the growth of bacteria has also been studied. Overnight broth culture was inoculated into the feather minimal medium. The flasks were placed in a shaking incubator at 120 rpm and incubated for 120 h at different temperatures of 25, 30, 35, 40, and 45°C. The pH of the medium was adjusted before autoclaving. Growth was measured by reading the absorbance at 620 nm at every 24 h interval against a control blank without inoculums.

2.6. Effect of Different Feather Concentration and Various substrates on Bacterial Growth

Various concentrations of feather meal and different substrates were added in culture media to identify the better growth and higher degradation of bacteria. Different concentrations of feather powder such as 0.5, 1.0, 1.5, and 2.0 g with the bacterial inoculums were used for the determination of bacterial growth.

Minimal medium was supplemented with 1% each of bovine serum albumin and gelatin and peptone, respectively, to study the effect of various substrates on growth. Each flask was inoculated with the isolated bacteria and incubated at 37°C for 120 h at 120 rpm. The growth was estimated at every 24 h interval by reading the absorbance at 620 nm against a control blank without inoculums.

2.7. Protein Estimation

The protein content of the cell free supernatant was determined by standard protocol [33] with bovine serum albumin as standard. One milliliter sample was mixed with 5 ml freshly prepared alkaline copper sulfate and then incubated at room temperature for 10 min. Then added 0.5 ml Folin-ciocalteau's reagent, incubated for 30 min and the absorbance were read at 660 nm against a reagent blank.

2.8. Estimation of Aminoacids

The concentration of free amino groups in the culture supernatant was determined by ninhydrin method [10] with glycine as standard.

2.9. Submerged Fermentation

Fermentation process was carried out following submerged fermentation method [34] using feather powder as substrate. The basal minimal medium consisted of 1% chicken feather powder as sole source of carbon and nitrogen. Further, fermentation tank was filled with 2 L of autoclaved medium. Fermentation was initiated by seeding the prepared inoculum and was carried out at 37°C, with pH 7.0 and 100 rpm. During the fermentation process, 10 ml media along with culture was siphoned from the tank at every 24 h interval for 120 h. The sample was centrifuged at 5000 rpm for 15 min, and

the supernatant was used for the estimation of protein and amino acid. The biomass was calculated using the culture pellet. A thin smear of the feather hydrolysates was made on a clean glass slide and the changes in the architecture and shape of the feather were observed under the light microscope (LABOMAD LX 300) at 100× magnification.

3. RESULTS

3.1. Isolation and Identification of Keratinolytic Bacteria

In the present study, 14 bacteria displayed keratinolytic properties and three bacteria demonstrated better activities were chosen for further studies. Based on the morphological, physiological, and biochemical tests [Table 1] and in agreement with the characteristic features described in Bergey's Manual of Systematic Bacteriology, the isolates were identified as *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus polymyxa*, *B. pumilus*, *Bacillus subtilis*, *B. megaterium*, *Glutamicibacter arilaitensis*, *Micrococcus luteus*, *Micrococcus* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas microphilus*, and *Serratia marcescens*, respectively. Among them, three bacteria such as *B. licheniformis*, *G. arilaitensis*, and *S. marcescens* exerted better activity and they were considered for further studies. Those three bacteria were also confirmed by their 16S rRNA gene sequence and the sequences have been deposited in the NCBI and obtained GenBank accession numbers MH063522.1, MH063920.1, and MH064150.1 with high matches similarity [Figure 1] respectively.

3.2. Effect of Different pH and Temperature on Bacterial Growth

Feather powder was used as the substrates for pH and temperature optimization, various culture parameters have been optimized to attain maximum feather degradation by the bacterial isolates. As the temperature increased, growth of bacteria was also increased. The growth of *G. arilaitensis* was high at pH 8.5 and 40°C, respectively. Similarly, for *B. licheniformis*, it was 8.0 and 40°C and for *Serratia marcescens*, it was 8.5 and 40°C, respectively. The amount of protein produced by *B. licheniformis* was high at pH 8.5 and 40°C, respectively. *G. arilaitensis*, it was 8.0 and 40°C

Table 1: Biochemical characterization of the isolated keratinolytic bacteria.

S. No	Biochemical Tests	Bacterial isolates													
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
1	Gram staining	+R	+R	+R	+R	+R	+R	-R	-R	-R	+C	+R	-R	+C	+R
2	Methyl red	-	+	+	+	-	+	-	+	-	-	-	-	-	-
3	Voges-proskauer Test	+	+	+	+	-	+	-	-	+	-	+	-	-	-
4	Starch hydrolysis	+	+	+	-	+	+	+	NR	+	NR	+	NR	+	+
5	Urease test	-	NR	NR	NR	NR	-	+	+	+	NR	NR	-	+	-
6	Citrate utilization	+	+	+	-	+	+	+	+	+	+	-	+	-	NR
7	Indole test	-	NR	-	-	NR	-	+	-	NR	NR	-	+	-	-
8	Casein hydrolysis	+	+	+	NR	+	+	NR	NR	+	+	+	NR	+	+
9	Oxidase test	NR	+	+	+	NR	NR	+	-	-	+	NR	NR	NR	-
10	Catalase test	+	+	+	+	+	+	NR	+	+	+	NR	NR	+	+
11	Mannitol salt agar test	NR	-	NR	NR	+	NR	NR	NR	+	NR	+	+	+	-

Identified organisms: I= *Bacillus subtilis*, II= *B. Cereus*, III= *B. licheniformis*, IV= *B. pumilus*, V= *B. megaterium*, VI= *B. thuriangiensis*, VII= *Pseudomonas microphilus*, VIII= *Proteus vulgaris*, IX= *Serratia marcescens*, X= *Micrococcus luteus*, XI= *Bacillus polymyxa*, XII= *Pseudomonas aeruginosa*, XIII= *Micrococcus SPe*, XIV= *Glutamicibacter arilaitensis*+ R = Positive Rod; + C=Positive Cocci, NR=No Results.

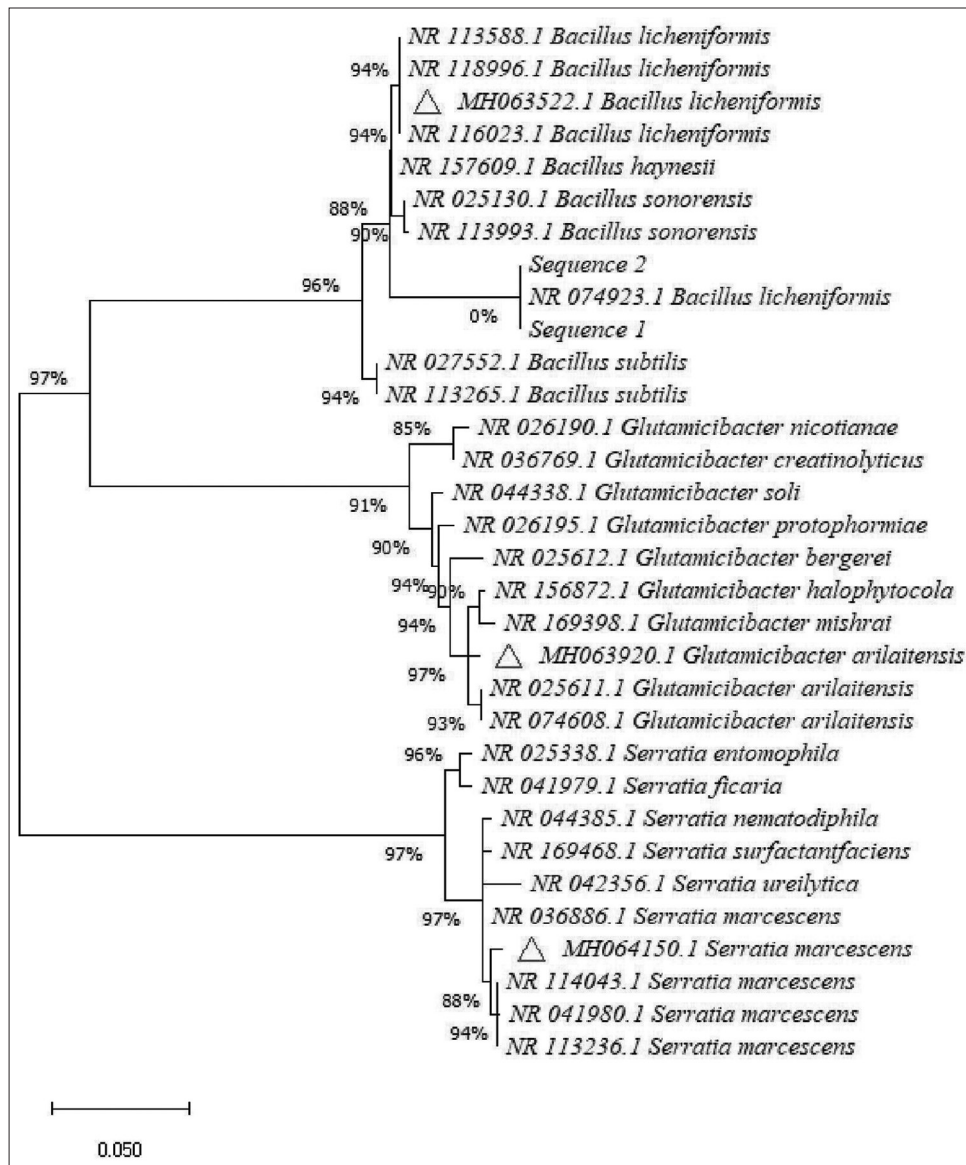


Figure 1: Phylogenetic tree based on 16S rRNA sequence of isolated bacteria. The branching pattern was generated by neighbor-joining method.

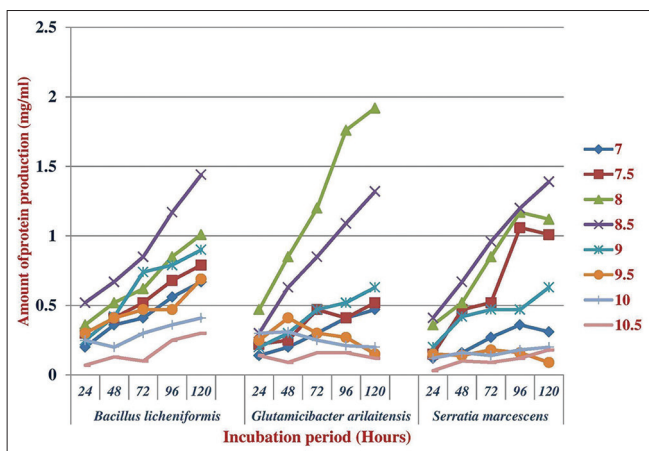


Figure 2: Effect on pH on the production of protein by the isolated feather degrading bacteria.

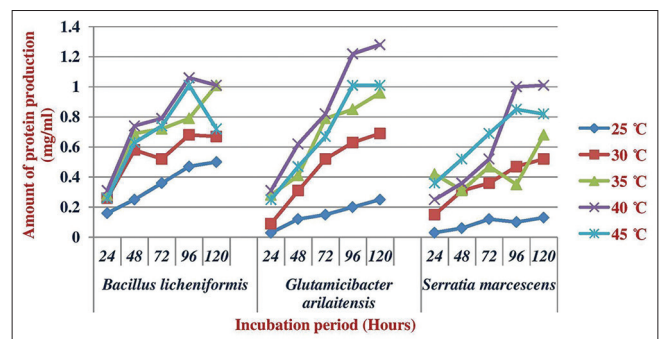


Figure 3: Effect of temperature on the production of protein by the isolated feather degrading bacteria.

[Figures 2 and 3] and, for *S. marcescences*, it was 8.5 and 45°C, respectively, which suggest that keratinase activity depends on the growth of bacteria.

3.3. Effect of Different Feather Concentration and Various substrates on Bacterial growth

The optimum feather concentration for growth of all the tested bacteria was 1 g/100 ml and the obtained results are depicted in Figures 4 and 5. Different species of microbes releases different amount of protein production and growth using various growth substrates. Subsequently, for the growth and maximum protein, production of *G. arilaitensis* and *S. marcescens* utilized gelatin and bovine serum albumin exhibited highest protein production of *B. licheniformis*. Among the various substrates provided, gelatin was the least preferred by *B. licheniformis* and *G. arilaitensis* and for *S. marcescens* peptone was the least preferred substrate.

3.4. Submerged Fermentation

The fermentation process was carried out using feather as the source of Carbon. The hydrolysate was tested at every 24 h interval for soluble amino acid, protein, and biomass and the results are tabulated [Tables 2-4]. The amount of amino acid and protein in the cell free supernatant was the best indicator for the progression of biodegradation. A thin smear of the feather hydrolysates was also prepared for every 24 h interval, examined under the microscope, and photographed. Feather rachis and barbs were ruptured into smaller fragments. Consequently, there was a lot of modification in the arrangement of barbs and rachis and architecture of the feather fragments [Figures 6-8]. Visual examination of unstructured feathers under the microscope at high magnification described the prominent variation in feather configuration at every 24 h interval. Considering the results, it has been concluded that, among the

three bacteria, better degradation of feathers was exhibited by *G. arilaitensis*.

4. DISCUSSION

Several million tons of feathers are generated every year by the poultry sectors across the globe which leads to troublesome environmental pollution and subsequent wastage of protein [10]. In this study, 14 keratinolytic bacteria were isolated from soil based on the growth, biomass, and protein content in the medium. The three better strains such as *B. licheniformis*, *G. arilaitensis*, and *S. marcescens* were chosen for further studies. Microorganisms utilize feathers as a source of nutrient for their growth by hydrolyzing keratin through keratinolysis, sulfitolysis, and proteolysis [35]. Optimization is notably an important criterion in the development of microbial process. Cost-effective optimization of culture conditions for keratinolytic bacteria is a challenge, and only few significant results were achieved for large scale process [36]. Furthermore, increasing in temperature causes the denaturation of enzyme and also microorganisms could not survive at high temperatures. This indicates that the isolates might be mesophiles and not favorable for enzyme production at high temperatures. These results are in accordance with results described by Sahoo *et al.* [37] who obtained optimum enzyme production at 40°C with *Bacillus weihenstephanensis*.

Chaisensaeng *et al.* [15] reported that the best temperature was between 40 °C and 45°C for the production of keratinolytic enzyme by bacilli (*B. licheniformis* and *Brevibacillus brevis*). *B. licheniformis* ALW 1

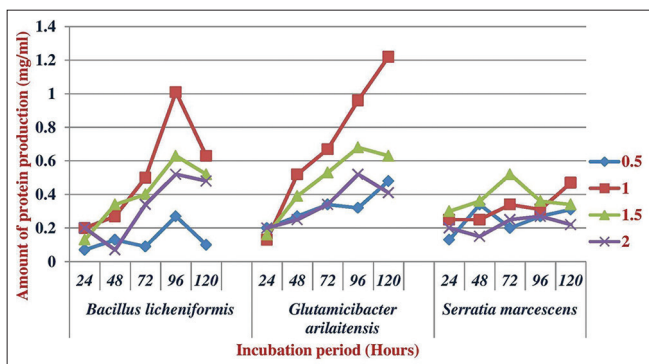


Figure 4: Effect of concentration on the production of protein by the isolated feather degrading bacteria.

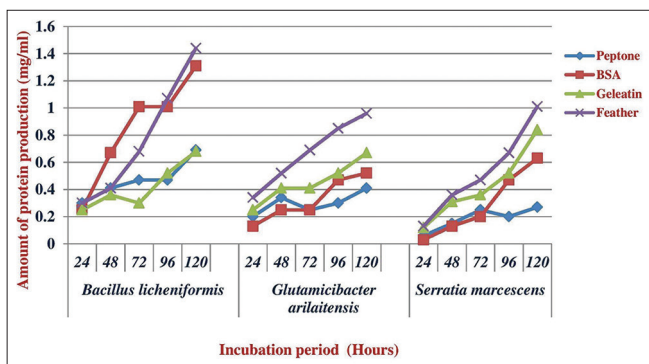


Figure 5: Effect of various substrates on the production of protein by the isolated feather degrading bacteria.

Table 2: The impact of *Bacillus licheniformis* on fermentation.

Hours	Dry feather weight (g)	Biomass (g)	Protein (mg/ml)	Amino acids (µg/ml)
24	1.24	0.002	0.57±0.11	0.063±0.011
48	0.67	0.016	0.55±0.08	0.10±0.028
72	1.01	0.103	0.41±0.03	0.125±0.037
96	0.44	0.012	1.45±0.03	0.218±0.019
120	0.62	0.008	0.58±0.10	0.180±0.018

Data represents mean±standard deviation of independent triplicate.

Table 3: The impact of *Glutamicibacter arilaitensis* on fermentation.

Hours	Dry feather weight (g)	Biomass (g)	Protein (mg/ml)	Aminoacids (µg/ml)
24	1.33	0.03	0.81±0.11	0.10±0.028
48	0.99	0.016	0.90±0.10	0.025±0.018
72	0.064	0.012	1.37±0.03	0.317±0.011
96	0.018	0.018	1.42±0.03	0.386±0.049
120	0.012	0.004	2.15±0.04	0.498±0.019

Data represents mean±standard deviation of independent triplicate.

Table 4: The impact of *Serratia marcescens* on fermentation.

Hours	Dry feather weight (g)	Biomass (g)	Protein (mg/ml)	Amino acids (µg/ml)
24	0.092	0.02	0.92±0.08	0.205±0.011
48	0.083	0.04	1.24±0.06	0.262±0.101
72	0.092	0.05	1.49±0.10	0.305±0.011
96	0.062	0.06	1.42±0.03	0.448±0.011
120	0.018	0.02	1.66±0.06	0.504±0.028

Data represents mean±standard deviation of independent triplicate.

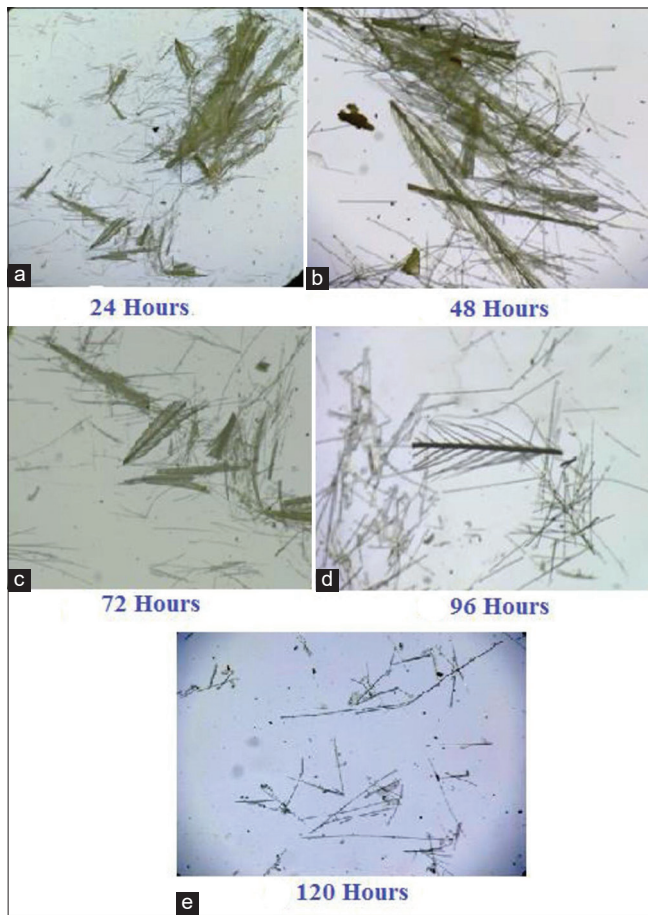


Figure 6: (a-e) Microscopic view of degraded feather during fermentation by *Bacillus licheniformis*.

produced keratinase that exhibited good stability over a range of pH (7–9) and temperature (50–60°C) and was able to degrade native feathers up to 63% in redox free system [38]. Similarly, Mosavi *et al.* [39] also reported maximum keratinase activity by *B. subtilis* isolated from poultry waste at pH 11 and 40°C on the 6th day of culture. A novel keratin degrading protease, keratinase BsKER71 from *B. subtilis* S1-4, was cloned and expressed under high temperature regime of 50–55°C in a wide range of pH 9 and 10 [40]. Furthermore, *B. licheniformis* optimally produced the enzyme at pH 10 and temperature 40°C and *Geobacillus stearothermophilus* produced keratinase at 55 °C and pH 7.0–8.0 [41].

The pH changes not only affect nature of the medium, substrates, and also affect growth and enzyme production by affecting enzymes transport across to the substrate so that growth in unsuitable conditions limits the microbial growth and enzyme production. The rate of microbial growth may be lower or higher than optimum due to the changes in three dimensional structure of proteins, affect the ionization of R groups of amino acids in active site or in other parts of the enzyme so that the enzyme changes shape and it decreases enzyme catalytic ability. Hence, change in pH is one of the significant characteristics of keratin degradation and the present research used the pH of the basal medium at 7–9 for better bacterial growth and stimulation of the enzyme. In this study, *G. arilaitensis* shows good degradation by producing more keratinase. Barman *et al.* [32] reported a synonymous strain *Arthrobacter* sp. which was a good candidate for keratinase production; however, reports are scarce on the feather degradation ability of *G. arilaitensis*. Most of the

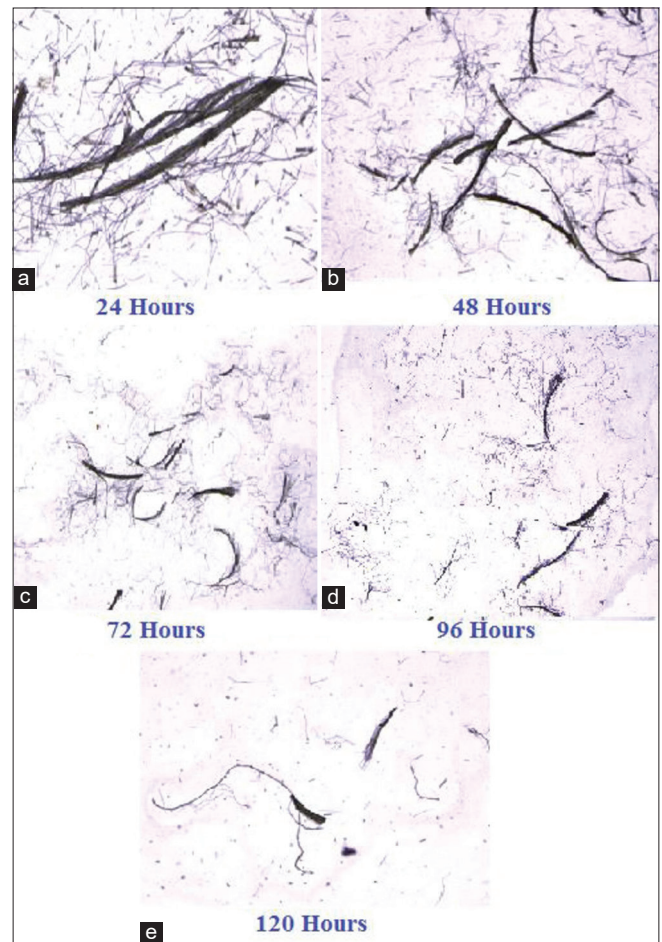


Figure 7: (a-e) Microscopic view of degraded feather during fermentation by *Glutamicibacter arilaitensis*.

keratinases are released extracellular and the enzyme was stable at pH 6–8 for *Bacillus* strains [42,43] and the keratinases produced by the *Bacillus* species might be active in neutral to basic pH conditions. *B. licheniformis* BBE11-1 and *Stenotrophomonas maltophilia* BBE11-1 efficiently degraded 50 g/L of chicken feather waste and produced large amounts of amino acids and antioxidant substances at a conversion rate of 70.0% [44].

Different mechanisms were proposed for microbial decomposition of keratin, in which cleavage of disulfide bonds before proteolytic breakdown is inherent [45]. The protease produced by *Arthrobacter arilaitensis* Re117 and *Serratia* sp. has high stability and purified with ion exchange chromatography technique [46–48]. Biodegradation of keratin under culture conditions is a process dependent on the microbial strain and type of keratinase. Keratinase shows variable keratinolytic activity toward different substrates. Moreover, the efficiency degradation rate linked with type of substrates and amount of cysteine of the substrates. *Aeromonas hydrophila* K12, *Chryseobacterium indologenes* A22, and *S. marcescens* P3 produced high amount of soluble proteins and formed thiol groups during keratinase production [37]. Khardenavis *et al.* [49] isolated *Serratia* HPC 1383 to enhance the degradative capacity to further shorten the time for digestion of feather waste to protein-rich animal feed by optimizing the kinetic contents of keratinase. Aarti *et al.* [50] reported that *G. arilaitensis* ALA4 produced amylase at 4°C with goat dung as substrate; these results indicate that *G. arilaitensis* has higher ability to degrade keratin from feathers. The present study is in agreement with

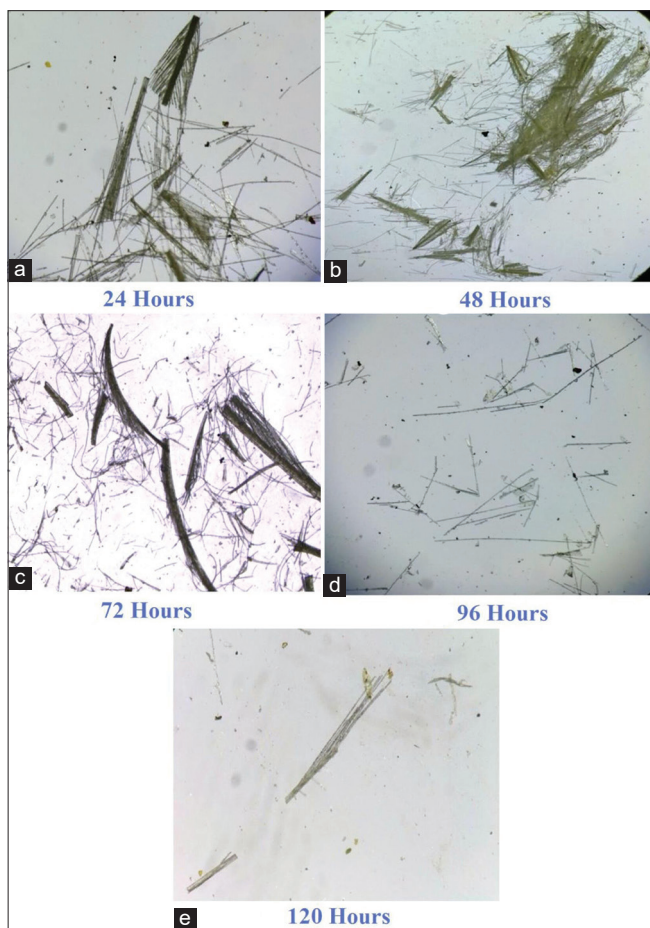


Figure 8: (a-e) Microscopic view of degraded feather during fermentation by *Serratia marcescens*.

their findings, wherein under optimized culture conditions keratinase production was high and degraded feathers in a better way.

Filamentous fungi and bacteria degrade feather by keratinase through sulfidolytic cleavage of cystine [18] and accumulation of reduced thiols due to direct reduction with specific reductase-like enzymes [51]. Vereza *et al.* [52] concluded that keratinase production and feather degrading activity *in vitro* are not only the properties of bacteria but also depend on other factors including keratin substrate, sterilization method, and culture conditions. High feather powder concentration above 1% may lead to increase in medium viscosity which results in oxygen limitation for bacterial growth. Cheng *et al.* [53] reported that 1% feather powder resulted in the highest keratinase activity for *B. licheniformis* PWD-1. *B. subtilis* S1 with 1% feather powder yielded better degradation activity [54]. Recently, the products of feather degradation have been used as a component of fertilizers which augment seed germination and growth rate [55]. The treatment of non-digestible biomass by these microorganisms may offer an industrial opportunity along with degradation of keratin [56]. The thermal alkaline microwave method, enzymatic method [57], and green technology based methods are most significant in the production of keratin hydrolysates which have enormous biotechnological significance [17].

5. CONCLUSION

G. arilaitensis degrades feather waste under optimized culture conditions in a better, safe, and ecofriendly way. Further, validation of

this research has a huge potential to employ this strain in the large scale degradation of chicken feather waste. Further studies are required to possibly convert the recalcitrant chicken feather waste into utilizable products for sustainable development.

6. ACKNOWLEDGMENT

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

11. PUBLISHER'S NOTE

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