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Research Article

Microbe Mediated Extraction of Keratin from *Gallus gallus domesticus* and its Role as a Fertilizer and in Poultry Feed

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Abstract: The poultry industry in Pakistan generates substantial feather waste, contributing to environmental contamination. This study aimed to isolate keratinolytic bacteria from chicken (*Gallus gallus domesticus*) feathers and soil samples. Twelve isolates exhibited proteolytic activity, forming clear zones on skim milk agar and growing efficiently on minimal agar, suggesting they belong to the Bacillus species. One promising strain, identified through 16S rRNA sequencing as *Pseudomonas aeruginosa*, demonstrated optimal feather degradation at pH 7.0-9.0 and temperatures of 37 °C and 45 °C. Protein content was measured as 4.8 mg/ml for chicken feathers and 5.43 mg/ml for quail feathers ($p < 0.05$). The FTIR spectrum of keratin hydrolysate for the chicken feathers was recorded within 4000-7000 cm^{-1}. Strain SC7 showed significant plant microbial interaction with tomato (*Solanum lycopersicum*), pea (*Pisum sativum*), and cucumber (*Cucumis sativus*), with dilutions of 100x (0.048 mg/ml) and 70x (0.068 mg/ml) promoting the growth of tomato, pea, and cucumber. When used as a feed additive, keratin significantly ($p < 0.05$) increased chicken body weight by 67.7% compared to controls. Liver function tests (ALT, AST, ALP, total protein, albumin, globulin, albumin/globulin ratio) showed decreased ALT and AST and increased ALP, total protein, albumin, and globulin in the experimental group compared to controls. Histopathology revealed no degenerative effects of keratin on chicken liver hepatocytes.

Keywords: *Bacillus, Pseudomonas aeruginosa,* Hydrolysate, Keratin Feed, FTIR, LFT, Histopathology, Chicken.

1. INTRODUCTION

Poultry industry is growing worldwide, and large amounts of excreta are coming from this industry [1]. With an average yearly rise of 1.7% since 2017, Pakistan's poultry meat production is expected to increase from 1.45 million metric tons in 2021 to 1.6 million metric tons by 2026 [2]. However, disposal of feathers is a major concern for the poultry industry and accumulation of this huge volume of the feathers results in environmental pollution and protein wastage [3]. It is well known that feathers are a very rich source of protein with β-keratin constituting 91 % of feather protein *Gallus gallus domesticus* [4]. Keratin is an insoluble protein molecule having very high stability and low degradation rate [5]. Based on secondary structural confirmation, keratins have been classified into α keratin (α-helix in hair and wool) and ß keratin (ß-sheets in feather) [6]. Many microbes like *Bacillus* spp., *Burkholdreia, Chryseobacterium, Pseudomonos, Microbacterium* spp. can degrade keratin by producing keratinase [7, 8]. Essential amino acids can be obtained from degraded feathers and act as precursor for the compounds which promote plant growth [9]. Replacing hazardous synthetic fertilizers with eco-friendly bio-fertilizers can be an alternative option for enhancing agricultural productivity [10]. A minor quantity of poultry feathers started to be used in other industrial applications such as clothing, insulation and bedding [11]. Presently, keratin hydrolysates are also in use in formulation of feed for livestock and fertilizer for agriculture industry [12]. There is scarce data available regarding isolation of bacteria, feather degradation, keratin estimation and its application as a fertilizer and feed in plants and animals respectively. The present study was conducted to isolate and characterize keratinolytic bacteria from the soil samples collected from dumpsite of a private poultry shed. This study is useful for converting poultry waste into value

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added products in the environment. The keratin hydrolysate was used as fertilizer for different plant species such as tomato (*Solanum lycopersicum*), Pea (*Pisum sativum*), and Cucumber (*Cucumis sativus*). Moreover, the keratin hydrolysate was also used as a feed additive for chicken.

2. MATERIALS AND METHODS

2.1. Collection of Samples and Preparation of Feather Powder from *Gallus gallus domesticus*

Feathers of *Gallus gallus domesticus* (Chicken) were collected from a local chicken shop along with dry soil from dumpsite of a private poultry shed in Jahman village near Lahore. The samples were collected in the month of May 2021 in a zipper plastic bag. After collecting feather samples these were autoclaved for 15 minutes at 121 ºC and dried in hot air oven for 4 hours at 50 ºC. The dried feathers were crushed in the grinder machine to obtain fine powder. The yield of keratin powder obtained from feathers was calculated by formula:

$$Yield = \frac{amount\ of\ feathers\ powder}{amount\ of\ feathers} \times 100$$

2.2. Isolation, Purification and Screening of Bacterial Isolates from Poultry Soil

Bacterial isolation from poultry feathers and soil was done by culture enrichment technique according to Kumar *et al.* [6]. Almost 30 different colonies were obtained and purified by quadrant streak. The purified colonies were further tested for their proteolytic activity and isolates were cultured on Skim milk Agar [13]. The plates were incubated for a week at 37 ºC and isolated based on clear zones. Almost twelve isolates were obtained and cultured on minimal growth media and named as SC1, SC2, SC3, SC4, SC5, SC6, SC7, SC8, SC9, SC10, SC11 and SC12.

2.3. Characterization and 16S rRNA Sequencing Bacterial Isolates

Selected bacterial isolates were characterized both morphologically and biochemically according to methods of Cappuccino and Sherman [14]. From all twelve isolates the genetic identification of only one isolate SC7 was carried out using 16S ribosomal gene sequence analysis using commercial services of IBM.

2.4. Preparation of Hydrolysate

Protein hydrolysate was prepared with the help of isolated microbes and biodegradation as carried out under controlled conditions at specific pH, and temperature. Later, this hydrolysate was used in the experiment as a protein source.

2.5. Effect of Temperatures and pH on Degradation of Feathers

All isolates were cultured at varying temperatures such as 25 ºC, 37 ºC and 45 ºC and pH 5.0, 7.0 and 9.0. The effect of isolates on the possibility of degradation was recorded for chicken and quail feathers. For this the isolated bacteria were incubated in LB-Broth at 37 °C for 24 hours. After incubation, Optical Density was taken at 600 nm and different parameters such as stereomicroscopy, protein estimation, FTIR, biofilm assay was monitored.

2.5.1. Stereomicroscopy

Samples of hydrolysate (1 ml) were analyzed using stereomicroscope before and after experiment completion. A stereomicroscope is a low-magnification optical instrument that uses two distinct optical channels to provide a three-dimensional picture of specimens. With improved depth perception, it is frequently employed to examine the surfaces of materials such as hydrolysate in this study.

2.5.2. Protein estimation

To study the Protein contents of degraded feathers (keratin obtained) by bacterial isolates, were determined using Bovine Serum Albumin (BSA) as the standard Lowry *et al*. [15]. The optical density (OD) was recorded at 750 nm. Optical Density is the amount of light absorbed by a sample as it passes through a medium. It is commonly used to measure the concentration of a solution and indicates the attenuation of light.

2.5.3. Biofilm assay

Biofilm formation of selected isolates was done

according to the methods of Qurashi and Sabri [16]. After 4 days, OD at 600 nm was observed for planktonic cells. Optical density of biofilm in term of adhered cells was recorded at 570 nm.

2.5.4. Fourier Transform Infrared (FTIR) spectroscopy analysis

The FTIR spectrum of the extracted keratin hydrolysate was analyzed for the detection of amide groups and functional groups. The Fourier Transform Infrared (FTIR) Spectrophotometer measures the infrared absorption of materials to identify and classify them. It operates on the premise that certain infrared light frequencies are absorbed by molecules, which causes their bonds to vibrate. Each substance's molecular fingerprint is provided by these absorption patterns. Keratin hydrolysates were sent to a private Laboratory for getting commercial services in Lahore, Pakistan and the result were obtained and noted.

2.6. Assessment of Plant Microbe Interaction (PMI)

The bacterial isolates (SC1, SC2, SC3, SC4, SC5, SC6, SC7, SC8, SC9, SC10, SC11 and SC12) were inoculated in autoclaved sterilized LB-Both and then incubated at 37 ºC for 24 hours. The incubated LB-Broth was then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded, and pellet was suspended in the normal saline. The seeds of tomato (*Solanum lycopersicum*), and Cucumber (*Cucumis sativus*) were sterilized with 0.1% $HgCl_2$ and soaked in pellet suspended in normal saline for 20 minutes. These seeds were placed in sterilized petri dishes, which have wet filter paper. The germination setup had 5 seeds of each plant/ isolate. These plates were kept in dark for 3 days until the germination. The dry weight and fresh weight of seedling was measured along with root and shoot length. Then plants were allowed to grow in sunlight. The growth was observed for the next 14 days, after that root and shoot length was again measured. Control seeds were not treated with pellets.

2.6.1. Use of keratin as a fertilizer

Keratin hydrolysate was used as a fertilizer and methods of Kshetri *et al*. [17] were followed with slight modifications. Keratin hydrolysate was filtered using a sieve of 0.2 mm to remove the undigested feathers. The seedlings were placed in dilutions of keratin hydrolysate for aqua culture and 5 plants per dilution were allocated to each set up. The certified seeds of tomato (*Solanum lycopersicum*), Pea (*Pisum sativum*), Cucumber (*Cucumis sativus*) were collected from Punjab Seed corporation Lahore Pakistan. The seeds were sterilized with 0.1% $HgCl_2$ and placed in petri dishes, with wet filter papers. The germination setup had 5 seeds of each plant. These plates were kept in dark for 3 days until the germination. Then plants were allowed to grow in sunlight, the growth was observed for the next 14 days. The control setup with 5 plants was placed in distilled water. The number of germinated seeds, root lengths, and shoot lengths were recorded and compared with both experimental and control setup.

2.7. Keratin as Poultry Feeds

Keratin was also used as a poultry feed as described by Fisinin *et al*. [18]. Feed was prepared in 50% w/v ratio. The feed was soaked in keratin hydrolysate and then air dried. There were two groups, one control and another was experimental. Birds of 10 days were placed in triplicates. The control group was fed by the regular poultry feed while experimental set was fed with feed that was soaked in keratin hydrolysate. The initial and final body weight were recorded. The body weight gain was calculated for both sets and compared.

2.7.1. Liver function test

The purpose of liver function test was to determine the liver metabolism, injury of a cell or response to the injury. The liver function test of chicks of both control and experimental setup was done. For this the blood samples of birds from both groups were collected and send to a private laboratory in Lahore, Pakistan. The parameters such as BILI (bilirubin), ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), TP (total protein), ALB (albumin), GLOB (globulin) and A/G (albumin/globulin) were recorded.

2.7.2. Histopathology of chick liver

Histopathology of liver was performed and for this purpose birds were slaughtered, and liver samples were collected, washed, labelled, and preserved

in 10% formalin. The histopathology test of liver samples was performed to check the efficacy of keratin hydrolysate that was obtained from microbial degradation of chicken feathers. For The samples were sent to private Laboratory for getting commercial services in Lahore, Pakistan and the result were obtained and noted.

3. STATISTICAL ANALYSIS

Statistical analysis was performed using mean, standard error, and least significant difference (LSD) tests using MS Excel 2016. Statistical analysis was performed for each parameter using standard error to calculate the mean and standard deviation. Analysis of variance (ANOVA) was conducted to assess significant differences, and the least significant difference (LSD) was applied for post hoc comparisons. All analyses were carried out using SPSS version 22.

4. RESULTS

4.1. Preparation of Feather Powder from *Gallus gallus domesticus* and Isolation of Bacteria

Feathers of *Gallus gallus domesticus* (Chicken) were finally crushed and changed to fine powder. While bacterial isolates from enrichment cultures were purified by streaking and then selected isolates were screened on skim milk agar for proteolytic activity. From 30 different bacterial colonies, that showed positive results in the form of clear zones on skim milk agar were purified by streaking and twelve isolates SC1, SC2, SC3, SC4, SC5, SC6, SC7, SC8, SC9, SC10, SC11 and SC12 were selected and characterized for their morphological and biochemical characteristics. It was noticed that all the isolated strains belonged to bacillus genus.

4.2. 16S rRNA Sequencing

Genetic identification and phylogenetic relationship of one of active bacterial isolates was described by using sequence analysis of 16S rRNA gene. The 16S rRNA gene sequence of SC7 785F strain was submitted to NCBI genbank and accession number was obtained which was ACCESSION OM846627. Phylogenetic tree based on 16S rRNA gene partial sequence of bacterial strain SC7 using the Neighbor-Joining method [19] was obtained. The results of 16S r RNA gene sequence (1457 bp) of SC7 showed closest (100%) similarity to *Pseudomonas* strain DSM 5425 and it was identified as *Pseudomonas sp.* strain, as shown in Figure 1.

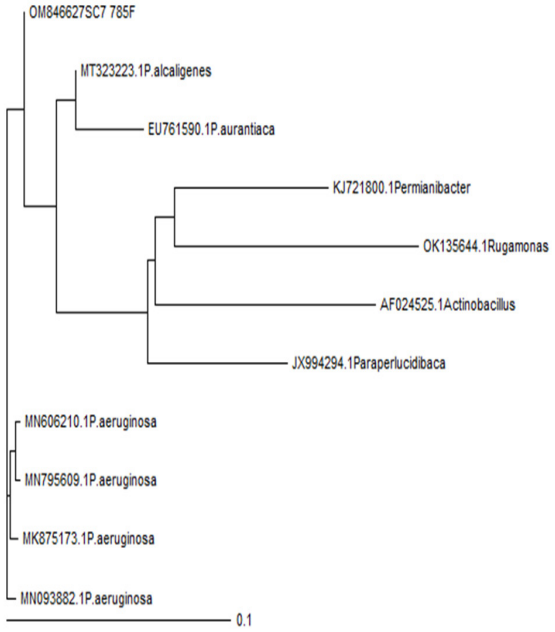

Fig. 1. Phylogenetic tree based on 16S rRNA gene partial sequence of bacterial strain SC7 using the Neighbor-Joining method [19].

4.3. Growth in Minimal Agar and Effect of Temperatures and pH on Bacterial Growth

The isolates were streaked on minimal agar plates and results were observed after 24 hours, all isolates showed noticeable growth in minimal media. Then the effect of varying pH conditions on bacterial growth was checked and results showed a general trend of significant ($p < 0.05$) increase in cell densities at pH 9.0 as compared to pH 5.0 and pH 7.0 with few exceptions. The analysis of cell densities showed that isolated SC7 showed the highest cell densities at pH 7.0 and pH 9.0 as compared to the results of other bacterial isolates and control treatment (Figure 2). Effect of varying temperature conditions on bacterial growth showed a significant ($p < 0.05$) increase in cell densities at temperature 37 ℃ and 45 ℃ as compared to temperature 25 ℃ for all bacterial isolates. The highest cell densities at different temperatures were shown by bacterial isolate SC8 that was significantly high ($p < 0.05$) as compared to other bacterial isolates and control treatment (Figure 3).

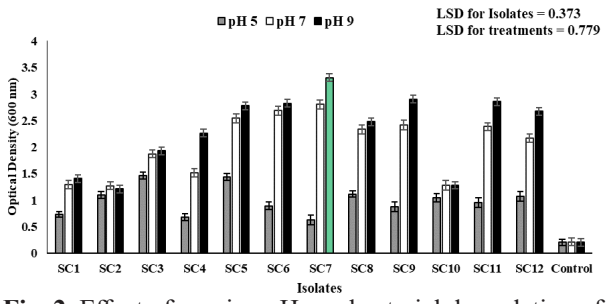

Fig. 2. Effect of varying pHs on bacterial degradation of feathers.

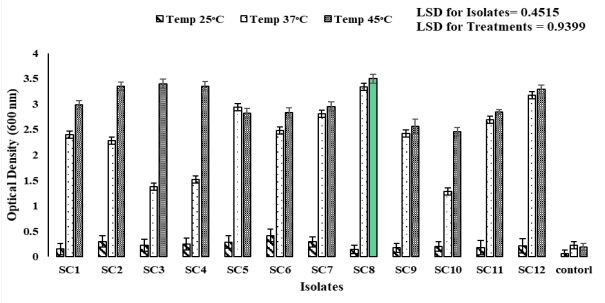

Fig. 3. Effect of Temperature on bacterial degradation of feathers.

4.4. Effect of Temperature and pH on Bacterial Degradation of Feathers

It has been observed that an increase in temperature resulted in increased feathers degradation with bacterial inoculation, and time of degradation was reduced as compared to control. An increase in temperature reduced the time for feathers degradation from 4 weeks to almost 3 weeks. Degradation of feathers was done in 3rd week at temperature 45 °C. While, at temperature 37 °C and 25 °C four and eight weeks were required for the feathers degradation in hydrolysate. Moreover, degradation of feathers completed at pH 5.0, 7.0 and 9.0 in 4th week in hydrolysate. Degradation of feathers substrate was found to be associated with significant increase ($p < 0.05$) in pH of the medium to alkalinity, thus serving as an indicator for the efficiency of degradation. Generally, an increased pH was observed toward completion of the experiment and media became alkaline despite adjusted pHs and temperatures.

4.5. Stereomicroscopy

The stereomicroscope was carried out at the start and end of the feathers' degradation, and it was noticed that degraded components were clearly visible in microscope as compared to control treatment.

4.6. Protein Estimation and Biofilm Assay

Bacterial Isolates obtained from *Gallus gallus domesticus* feathers and soil showed positive biofilm formation in 4 days at temperature 45 °C and pH 9.0. Optical density of biofilm in terms of adhered cells were recorded at 570 nm. The highest optical density was recorded by bacterial isolates SC7 and SC8 (Figure 4). The protein content of *Gallus gallus domesticus* feathers at 45 °C was 4.64 mg/ml and 4.8 mg/ml for bacterial isolates SC7 and SC8, respectively (Figure 5). At pH 9.0 protein content was 5.5 mg/ml and 5.43 mg/ml for bacterial isolates SC7 and SC8, respectively (Figure 6).

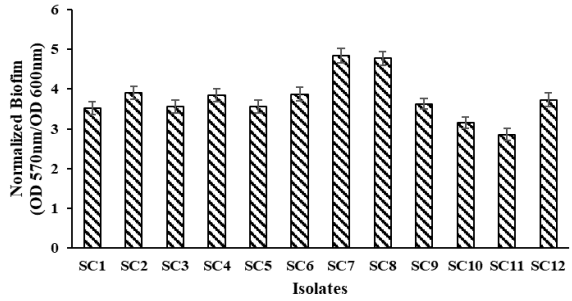

Fig. 4. Optimized biofilm bacterial isolates collected from Chicken feathers (*Gallus gallus domesticus*) at pH 9.0 and temperature 45 °C.

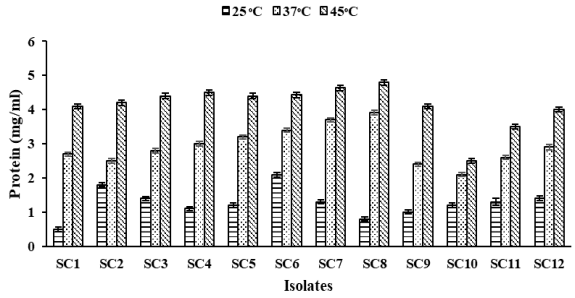

Fig. 5. Amount of protein content estimated from Chicken (*Gallus gallus domesticus*) feather at different temperatures.

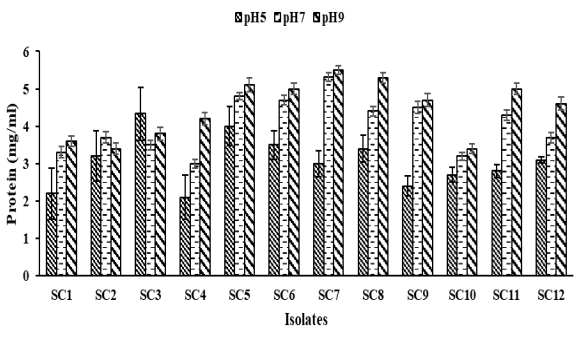

Fig. 6. Amount of protein content estimated from Chicken (*Gallus gallus domesticus*) on different pH.

4.7. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The FTIR spectrum was observed at 4000-700 cm^{-1} for both feathers. For keratin from chicken (*Gallus gallus domesticus),* the absorption peak range between 3500-3100 cm^{-1} (Figure 7) indicating O-H stretching. The absorption peak at range between 3000-2900 cm^{-1} showed C-H stretching represented amid-III group, peaks range between 1700-1600 cm^{-1} showed C=O stretching represented amid-I group, peaks range between 1450-1400 cm^{-1} showed N-H group represented amid-II group. Peaks at 877, 1085, 1043 cm^{-1} ranged between 1100-800 cm^{-1} showed S=O stretching represented cysteine.

4.8. Plant Microbe Interaction (PMI)

The plant microbe interaction experiments were carried out to check the efficacy of isolates in germination on tomato (*Solanum lycopersicum*) and cucumber (*Cucumis sativus)* seeds. Plant microbe interaction showed that there was a general trend of significant ($p < 0.05$) increase in germination and growth of tomato and cucumber seeds as compared to control. The bacterial isolates SC7 and SC8 were highly significant ($p < 0.05$) for the tomato and cucumber seed germination and growth as compared to other bacterial isolates and control (Table 1). The bacterial isolates SC8 was significant ($p < 0.05$) for cucumber seeds in reference to fresh and dry weight and for tomato seed SC8 was significant for fresh weight and SC7 and SC8 were significant for dry weight as compared to other bacterial isolates and control (Table 2).

4.9. Keratin as Fertilizer

Keratin as fertilizer (at 50x, 70x and 100x) showed positive results for all 3 plants, tomato (*Solanum lycopersicum*), pea (*Pisum sativum*), and cucumber (*Cucumis sativus)*. Keratin as fertilizer showed significance ($p < 0.05$) growth at 100x having 0.048 mg/ml keratin protein for root length, shoot length, plant length as compared to other treatment and control (Table 3). 100x (0.048 mg/ml) and 70x (0.068 mg/ml) dilution showed significant ($p < 0.05$) fresh weight and dry weight as compared to other treatments and control (Table 4).

4.10. Keratin as Poultry Feeds

It was observed that keratin as feed gave effective results on the chicken weight. The weight of experimental chicks was greater as compared to control group and significant differences at ($p < 0.05$) was noticed in experimental group with overall weight gain of 67.7% while, it was only 50.9% for the control group (Table 5).

4.11. Liver Function Test

Liver function test was performed for both experimental and control groups and results showed

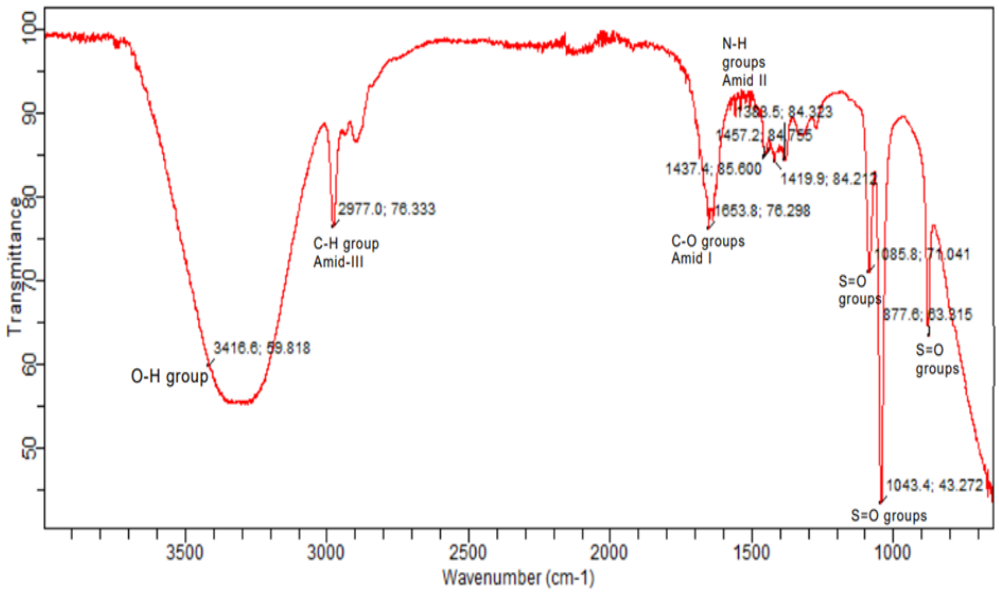

Fig. 7. Fourier Transform Infrared analysis of keratin hydrolysate.

that level of Bilirubin was 0.8 mg/dl for all chicks. While a decrease in ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels were noticed for experimental groups as compared to control. Levels of ALT and AST were 16.6 U/L and 187.3 U/L for the experimental group and 29.6 U/L and 214.6 U/L for the control group, respectively. While an increase in ALP (alkaline phosphatase) and total blood protein levels were noticed for the experimental group that were 3777.3 U/L and 4.2 g/dl as compared to control group that were 15581 U/L and 4.9 g/dl, respectively. Levels of Albumin and globulin were also determined and increased levels were noticed for experimental group as compared to control. The levels were 1.56 g/dl and 2.63 g/dl for the control while, 1.8 g/dl and 13.9 g/dl for the experimental group for albumin and globulin, respectively. The ratio of albumin/globulin for control on average was 0.59 and for experimental the ratio of albumin/globulin on average was 1.47 (Table 6).

Table 1. Effect of plant microbe interaction on percentage germination and length parameters.

Isolates	Root length (cm)	Shoot length (cm)	Plant length (cm)	Germination %
Tomato (*Solanum lycopersicum)*				
SC1	0.723 ± 0.09	0.78 ± 0.14	2 ± 0.21	46.6 ± 0.70
SC2	1.089 ± 0.22	2.84 ± 0.11	2.86 ± 0.07	33.3 ± 0.98
SC3	0.88 ± 0.04	2.48 ± 0.21	2.68 ± 0.14	66.6 ± 0.77
SC4	1.15 ± 0.22	3.56 ± 0.32	4.6 ± 0.21	66.6 ± 0.91
SC5	1.12 ± 0.04	2.92 ± 0.21	4.34 ± 0.21	66.6 ± 0.63
SC6	1.4 ± 0.13	2.32 ± 0.14	2.6 ± 0.14	40 ± 0.70
SC7	2.64 ± 0.17	3.94 ± 0.21	5.16 ± 0.19	86.6 ± 0.56
SC8	2.36 ± 0.28	3.78 ± 0.12	5.92 ± 0.16	80 ± 0.91
SC9	0.94 ± 0.33	3.24 ± 0.21	4.06 ± 0.20	60 ± 0.98
SC10	0.354 ± 0.21	0.68 ± 0.04	1.29 ± 0.33	53.3 ±0.63
SC11	1.34 ± 0.35	1.38 ± 0.14	2.72 ± 0.11	66.6 ± 0.84
SC12	0.9 ± 0.21	3.52 ± 0.21	4.42 ± 0.14	73.3 ± 0.70
Control	0.414 ± 0.08	0.46 ± 0.05	0.81 ± 0.09	33.3 ± 0.98
LSD for treatment= 1.42		LSD for Isolates= 0.68		
Cucumber (*Cucumis sativus)*				
SC1	1.12 ± 0.16	4.45 ± 0.21	5.57 ± 0.14	53.3 ± 0.70
SC2	1.18 ± 0.14	2.7 ± 0.14	3.88 ± 0.35	40 ± 0.21
SC3	2.97 ± 0.42	4.28 ± 0.28	7.25 ± 0.21	53.3 ± 0.70
SC4	4.02 ± 0.35	4.19 ± 0.21	8.22 ± 0.28	53.3 ± 0.84
SC5	2.91 ± 0.49	4.25 ± 0.35	7.16 ± 0.14	60 ± 0.98
SC6	2.82 ± 0.35	1.52 ± 0.21	4.34 ± 0.21	53.3 ± 0.70
SC7	4.22 ± 0.21	7.84 ± 0.18	12.08 ± 0.28	73.3 ± 0.42
SC8	4.24 ± 0.28	7.6 ± 0.21	11.85 ± 0.14	66.6 ± 0.21
SC9	3.35 ± 0.35	3.32 ± 0.14	6.67 ± 0.21	46.6 ± 0.84
SC10	0.8 ± 0.14	4.96 ± 0.28	5.96 ± 0.28	40 ± 0.28
SC11	3.76 ± 0.11	3.97 ± 0.35	7.73 ± 0.14	53.3 ± 0.21
SC12	3.58 ± 0.21	6.36 ± 0.21	9.94 ± 0.28	60 ± 0.35
Control	1.61 ± 0.21	2.69 ± 0.14	4.3 ± 0.21	40 ± 0.28
LSD for treatment 1.88		LSD for Isolates 0.90		

Table 2. Effect of plant microbe interaction on fresh weight and dry weight.

Isolates	Fresh weight (g)	Dry weight (g)
Tomato (*Solanum lycopersicum)*		
SC1	0.4729 ± 0.21	0.0332 ± 0.01
SC2	0.2749 ± 0.14	0.0275 ± 0.03
SC3	0.6688 ± 0.21	0.0561 ± 0.02
SC4	0.7236 ± 0.14	0.1259 ± 0.14
SC5	0.7759 ± 0.35	0.2955 ± 0.07
SC6	0.8224 ± 0.14	0.3952 ± 0.02
SC7	0.8517 ± 0.35	0.5948 ± 0.04
SC8	0.9076 ± 0.28	0.743 ± 0.14
SC9	0.7935 ± 0.14	0.453 ± 0.28
SC10	0.3758 ± 0.28	0.002 ± 0.001
SC11	0.8636 ± 0.42	0.45 ± 0.07
SC12	0.7049 ± 0.14	0.252 ± 0.06
Control	0.2679 ± 0.12	0.0133 ± 0.003
LSD for treatment 0.19		LSD for Isolates 0.08
Cucumber (*Cucumis sativus)*		
SC1	0.3 ± 0.28	0.024 ± 0.02
SC2	0.34 ± 0.14	0.02 ± 0.04
SC3	0.32 ± 0.42	0.02 ± 0.11
SC4	0.46 ± 0.35	0.027 ± 0.03
SC5	0.86 ± 0.16	0.009 ± 0.007
SC6	0.53 ± 0.21	0.013 ± 0.004
SC7	0.81 ± 0.16	0.036 ± 0.04
SC8	1.46 ± 0.21	0.09 ± 0.03
SC9	0.37 ± 0.14	0.008 ± 0.007
SC10	0.28 ± 0.28	0.008 ± 0.003
SC11	0.27 ± 0.14	0.008 ± 0.004
SC12	0.27 ± 0.35	0.005 ± 0.002
Control	0.06 ± 0.02	0.03 ± 0.04
LSD for treatment 0.50		LSD for Isolates 0.2

Table 3. Effect of Keratin hydrolysate as fertilizer on root, shoot, and plant length.

Dilutions	Root length (cm)	Shoot length (cm)	Plant length (cm)
Tomato (*Solanum lycopersicum)*			
10x	1.2 ± 0.14	0.62 ± 0.14	1.82 ± 0.22
20x	0.56 ± 0.15	1.7 ± 0.07	2.26 ± 0.28
30x	1.28 ± 016	2.28 ± 0.21	3.56 ± 0.14
50x	1.8 ± 0.14	2.44 ± 0.28	4.24 ± 0.16
70x	2.2 ± 0.14	2.22 ± 0.15	4.42 ± 0.15
100x	5.2 ± 0.07	3.43 ± 0.28	8.9 ± 0.28
Control	0.55 ± 0.07	1.72 ± 0.14	2.27 ± 0.19
LSD for treatment 1.54			
Pea (*Pisum sativum)*			
10x	1.42 ± 0.14	2.36 ± 0.14	3.78 ± 0.15
20x	1.45 ± 0.21	1.76 ± 0.28	3.21 ± 0.21
30x	1.33 ± 0.21	3.18 ± 0.35	4.51 ± 0.28
50x	1.68 ±0.15	3.48 ± 0.28	5.14 ± 0.25
70x	1.81 ± 0.21	4.89 ± 0.21	6.69 ± 0.28
100x	3.05 ± 0.12	5.38 ± 0. 35	8.45 ± 0.20
Control	1.13 ± 0.09	1.3 ± 0.14	2.43 ± 0.28
LSD for treatment 1.37			
Cucumber (*Cucumis sativus)*			
10x	1.76 ± 0.16	0.61 ± 0.14	2.37 ± 0.35
20x	1.78 ± 0.26	0.92 ± 0.57	2.7 ± 0.21
30x	2.07 ± 0.04	2.4 ± 0.28	4.29 ± 0.21
50x	4.3 ± 0.28	2.35 ± 0.35	5.16 ± 0.42
70x	5.61 ±0.20	3.74 ± 0.18	9.35 ± 0.14
100x	6.2 ± 0.21	6.5 ± 0.35	12.7 ± 0.21
Control	1.1 ± 0.35	2.4 ±0.31	3.5 ± 0.28
LSD for treatment 2.2			

10x (keratin 0.48mg/ml) 20x (024mg/ml) 30x (0.09mg/ml) 50x (0.09 mg/ml) 70x (0.068mg/ml), 100x (0.048 mg/ml)

4.12. Histopathology Test of Liver Chicken

The slides of stained liver tissue were observed in a light microscope under 100X lens, Figure 8 shows the histopathology of the chicken in control group; while Figure 9 presents the histopathology of liver chicken in experimental group. Keratin seemed to have no degenerative effect on the liver as hepatocytes were healthy. The cellular structure of hepatocytes was found same in both control and experimental groups except difference in cell size that was 24 µm in experimental and 26 µm in control group. The slight reduction in liver cell size in keratin-fed chickens suggests improved nutrient utilization, supporting healthy growth without liver stress.

5. DISCUSSION

Keratin present in feathers is highly difficult to degrade but there are some microorganisms that produce keratinase enzymes, which could

Table 4. Effect of Keratin hydrolysate as fertilizer on fresh and dry weight of different plants.

Dilutions	Fresh weight (g)	Dry weight (g)
Tomato (*Solanum lycopersicum)*		
10x	0.0299 ± 0.01	0.0243 ± 0.02
20x	0.0379 ± 0.02	0.018 ± 0.04
30x	0.0858 ± 0.01	0.0467 ± 0.03
50x	0.3045 ± 0.07	0.0869 ± 0.01
70x	0.514 ± 0.21	0.0965 ± 0.02
100x	0.9032 ± 0.07	0.3964 ± 0.07
Control	0.2729 ± 0.21	0.0188 ± 0.02
LSD for treatment 0.34		
Pea (*Pisum sativum)*		
10x	0.12 ± 0.05	0.009 ± 0.002
20x	0.11 ± 006	0.011 ± 0.004
30x	0.07 ± 0.02	0.013 ± 0.004
50x	0.11 ± 0.06	0.024 ± 0.007
70x	0.15 ± 0.04	0.022 ± 0.009
100x	0.15 ± 0.01	0.049 ±0.01
Control	0.06 ± 0.02	0.007 ± 0.005
LSD for treatment 0.04		
Cucumber (*Cucumis sativus)*		
10x	0.32 ± 0.14	0.022 ± 0.02
20x	0.32 ± 0.21	0.02 ± 0.02
30x	0.37 ± 0.30	0.01 ± 0.02
50x	0.49 ± 0.14	0.033 ± 0.01
70x	0.54 ± 0.21	0.031 ± 0.02
100x	0.76 ± 0.13	0.018 ± 0.02
Control	0.06 ± 0.04	0.08 ± 0.03
LSD for treatment 0.33		
10x (keratin 0.48mg/ml) 20x (024mg/ml) 30x (0.09mg/ml) 50x (0.09 mg/ml) 70x (0.068mg/ml), 100x (0.048 mg/ml)		

efficiently degrade this resistant keratin [20]. In this study, the bacterial isolates were isolated from the soil of poultry dump site, purified by streaking and screened on skim milk agar for determining proteolytic activity. Similar methods for isolation

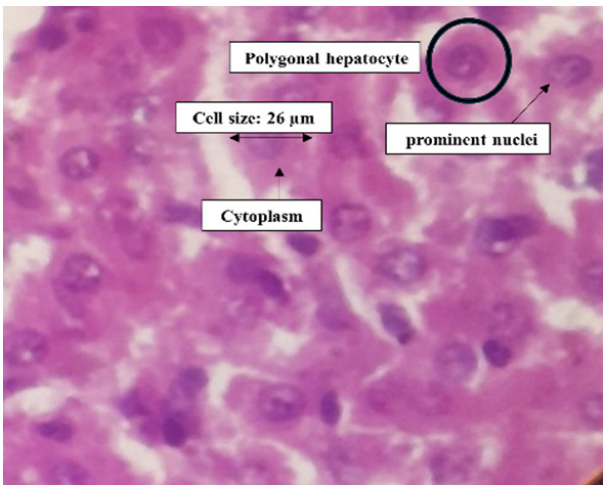

Fig. 8. Histopathology of the chicken in control group.

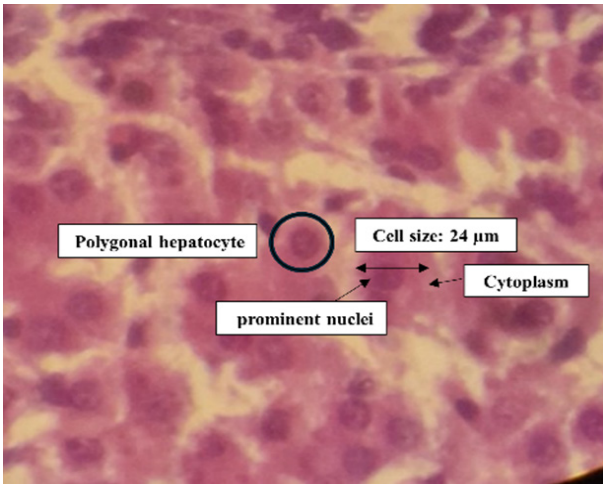

Fig. 9. Histopathology of liver chicken in experimental group.

and screening from poultry dump site were investigated previously [6, 20, 21]. The powder of *Gallus gallus domesticus* feathers was white color with powder yield 50%. By identification based on morphological, cultural and microscopic characteristics, the identity of the isolates was determined to be *Bacillus spp*. The recorded results are in line with previous findings about keratinolytic organisms which are found to *Bacillus spp.* [3, 12, 22-25].

Taxonomic affinities based on 16S ribosomal RNA gene sequence were analyzed using commercial service of gene sequencing (IBM) due to its reliable and trustworthy results. Results of 16S ribosomal RNA gene sequence were submitted in gene bank NCBI. Results showed bacterial

Table 5. Effect of Keratin hydrolysate as feed on chicken weight.

	Control group (without Keratin feed)			Experimental group (with Keratin feed)		
Sr. no.	**Initial weight (g)**	**Final weight (g)**	**% increase in weight (g)**	**Initial weight (g)**	**Final weight (g)**	**% increase in weight (g)**
Chick 1	76	94	23.6	90	148	64.4
Chick 2	51	77	50.9	93	156	67.7
Chick 3	76	86	13.1	76	101	32.8
LSD for treatments = 19.44, LSD for chicks = 15.87						

Table 6. Levels of different parrameters in liver function test.

Sr. no.	**BILI mg/dl**	**ALT U/L**	**AST U/L**	**ALP U/L**	**TP g/dl**	**ALB g/dl**	**GLOB g/dl**	**A/G**
Control chicks	0.53 ± 0.12	29.6 ± 3.37	214.6 ± 4.01	15581 ± 775.3	4.2 ± 0.05	1.56 ± 0.05	2.63 ± 0.03	0.59 ± 0.02
Experimental chicks	0.53 ± 0.12	16.6 ± 2.5	187.3 ± 4.28	3777.3 ± 591.8	4.9 ± 0.11	1.8 ± 0.17	13.9 ± 0.15	1.47 ± 0.01

BILI (bilirubin), ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), TP (total protein), ALB (albumin), GLOB (globulin), A/G (albumin/globulin).

homology with *Pseudomonas aeruginosa* bacterial identification on basis of 16S ribosomal RNA gene sequence is reliable for the identification of bacterial isolates. Comparison of 16S ribosomal RNA gene sequence revealed that these bacteria are diverse and predominated in ecological nations.

The keratinase enzyme production from *Pseudomonas aeruginosa* from 122 U/ml to 150 U/ml in organic and inorganic conditions was reported by Moonnee *et al.* [26]. The microbial strains which are isolated from waste of poultry and are best for the chicken feathers degradation are *Bacillus sp., Kocuria sp., Pseudomonas sp., and Fervidobacterium sp.* [27].

Effects of physiological conditions, pH and temperature, on bacterial growth were checked and results showed that there was a general trend of significant ($p < 0.05$) increase in cell densities at pH 9.0 and temperature 45 °C as compared to pH 5.0 and 7.0 and temperatures 25 ℃ and 37 ℃. Feather substrate degradation was established to be correlated with significant ($p < 0.05$) rise in pH of the medium towards alkalinity, thus giving an indication of the efficient degradation. These observations are in line with previous findings wherein keratinolytic bacteria have been reported to show optimal growth at thermophilic temperatures [28-30]. The degradation duration decreased at 45 ℃ as it was completed in 2 weeks instead of 4 weeks like at 37 ℃.

The keratin protein concentration in chicken feathers was found to be significant ($p < 0.05$) at pH 9.0 and temperature 45 ℃ which was 4.8 mg/ml and 5.5 mg/ml, respectively. There was an increase in soluble keratin protein content with an increase in pH of medium [30]. Sivakumar and Raveendran [32] estimated the keratin protein to be 414 µl/ml from chicken feathers degraded by *B. stubtilis*. An estimated keratin protein from chicken feathers degraded by *B. licheniformis* to be 8.28 mg/ml at 40 °C and pH 10.0 was reported in a study [33].

All 12 bacterial isolates obtained from *Gallus gallus domesticus* showed positive biofilm formation in 4 days at a temperature of 45 ℃ and pH 9.0. The biofilm formation by keratinolytic microbe increases the amount of keratin in extraction and promotes the degradation rate [34].

The FTIR analysis of keratin hydrolysate was observed between 4000-700 cm^{-1} wavelengths. O-H stretching absorption peak range was 3500-3100 cm^{-1}. Amid group III having C-H stretching was indicated by absorption peaks 3000-2900 cm^{-1}. Amid group I have C=O stretching was indicated by absorption

peaks 1450-1400 cm^{-1}. Amid group II having N-H bending was indicated by absorption peaks 1450-1400 cm^{-1}. Cysteine, S=O, stretching was observed at peaks ranging from 1100-800 cm^{-1}. The results of this study are in accordance with observations made by previous researchers [25, 33-37], who also found the FTIR spectrum range to be 4000-400 cm^{-1} and similar peaks for the functional groups.

Plant microbe interaction indicated that there was a general trend of significant ($p < 0.05$) increase in germination and growth of tomato and cucumber seeds as compared to control. *B. subtilis PF1* and metabolites that were released due to degradation of chicken feathers can be applied as nitrogen fertilizer for plants [38]. While *S. maltophilia R13* could be used as biocontrol agent and could also affect the fertilization of soil [39]. Similarly, *Bacillus cereus* could not only degrade chicken feathers but also improve plant growth development and is an effective plant growth promoting rhizo-bacteria [40].

Keratin as fertilizer showed significance ($p < 0.05$) growth at 100x having 0.048 mg/ml keratin protein for root length, shoot length, plant length fresh and dry weight as compared to other treatment and control. The optimal keratin concentration in feathers hydrolysate for seed germination is 0.1 mg/ml or below 0.1 mg/ml [41]. The compost was prepared from degraded chicken feathers and found out that it could be an economic source of nitrogen fertilizer [42]. The application of chicken feather hydrolysate to gram seeds resulted in early germination of seeds and higher growth of plant as compared to control [43].

Keratin hydrolysate increases the body weight of broiler chicken up to 9-10% as compared to fishmeal. The inclusion of feather meals in feed of broiler chicken had no negative effect on birds and it also reduces feed cost [44]. The increase in live weight of chicken to be 8.6% ($p < 0.001$) as compared to control [45]. It also increases the productivity of broiler but also higher antioxidant meat. To sum up, the present findings showed the efficacy of keratin degrading bacteria in poultry waste utilization into value added products.

The parameters of liver function tests can be used to determine cell injury of response to such an injury [46]. Blood activity of *Gallus gallus domesticus* can be determined by liver function tests [47].

The bilirubin was 0.8 mg/dl for both experimental and control chicks. The ALT (alanine aminotransferase) level was reduced for experimental setup as compared to control setup. For experimental the ALT level on average was 16.6 U/L while the control had 29.6 U/L. The AST (aspartate aminotransferase) level was reduced for experimental setup as compared to control setup. For experimental the AST level on average was 187.3 U/L while the controlled had 214.6 U/L. The ALP (alkaline phosphatase) level was reduced for experimental setup as compared to control setup. For experimental the ALP level on average was 3777.3 U/L while the control had 15581 U/L. Total protein was increased for experimental setup as compared to control setup. For experimental the total protein level on average was 4.9 g/dl while the control had 4.2 g/dl. The albumin was increased for experimental setup as compared to control setup. For experimental the albumin level on average was 1.8 g/dl while the control had 1.56 g/dl. Globulin was increased for experimental setup as compared to control setup. For experimental, the globulin level on average was 13.9 g/dl while the control had 2.63 g/dl. The ratio of albumin/globulin was increased for experimental setup as compared to control setup. For experimental the albumin/globulin on average ratio was 1.47 while the control had 0.59.

The normal values of level of ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), Total protein, Albumin, Globulin and Albumin/ Globulin ration in chicken were described as liver of chicken is 0.9 µg/kg/min [48]. The normal range of ALT in chicken is 15.3-55.3 U/L [49]. The AST normal range for chicken is 70-220 U/L and ALP normal range for chicken is 568-8831 U/L [50]. The normal range of total protein to be 33-55 g/l, albumin to be 13-28 g/L and globulin to be 15-41 g/l reported for chicken [51]. [In another study the albumin/globulin ratio for chicken from day 4 to day 46 was reported as 0.59-0.85 [52] and the albumin/globulin ratio for chicken from day 14 to day 42 was also depicted as 1.50-1.72 [53].

The histopathology results of chick liver indicated that keratin had no degenerative effect on the liver as hepatocytes were healthy. Xu *et al*. [54] found that nanoparticles of keratin are useful for the delivery of drugs to the kidney and liver. The use of chicken feathers for the formation of hydrogel

based on keratin also reported by Tang *et al*. [55]. These results showed keratin to be an effective drug carrier and had good hemostatic effect on tail and liver injury of mouse. Similarly, studies with nanoparticles prepared from keratin of chicken feathers showed an increased rate in coagulation in liver scratch model exhibiting their effectiveness in bio medical application [56, 57].

6. CONCLUSIONS

Avian feathers can be degraded by *Bacillus* strains isolated from soil at optimum temperature (45 °C) and pH (9), particularly within 3 weeks. This can help with the rapid removal of feathers' waste that is coming from the poultry industry. The keratin hydrolysate obtained as a result could be a cheaper source of fertilizer and an alternative of chemical fertilizers as it has potential for the growth of plants. The keratin hydrolysate can also be used as feed additive, and it helped for the better productivity of chickens in poultry industry as an increase in body weight and healthier chicken were observed during the study who were fed with keratin hydrolysate.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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