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EXTRACTION OF KERATIN FROM CHICKEN FEATHERS AND ITS POTENTIAL APPLICATIONS IN POULTRY FEED AND ORGANIC FERTILIZER

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ABSTRACT

Millions of tons of chicken feathers are produced globally each year by the poultry industry. The present study was designed to isolate keratinolytic bacteria from soil and feathers of *Gallus gallus domesticus* (chicken). Samples were collected from a private poultry farm. Qualitative analysis of keratin-degrading bacteria was carried out using the skim milk agar assay, and twelve bacterial isolates were obtained from *Gallus gallus domesticus* feathers. Bacterial cell densities were highest at 37°C and 45°C and at pH values ranging from 7.0 to 9.0. All twelve *Gallus gallus domesticus* isolates efficiently degraded *Columba livia domestica* feathers. The results showed that the protein content of *Columba livia domestica* feathers was 7.818 mg/mL, whereas that of *Gallus gallus domesticus* feathers was 3.949 mg/mL. The FTIR spectra of keratin hydrolysate from *Gallus gallus domesticus* and *Columba livia domestica* ranged from 700 to 4000 cm^{-1}. Among the isolates, SHC4 and SHC12 showed the best growth-promoting effects on fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*) and spinach (*Spinacia oleracea*) plants. Keratin hydrolysate used as a fertilizer significantly enhanced plant growth ($p < 0.05$) at 100×, 70× and 50× dilutions. Keratin used as poultry feed showed a maximum body-weight gain of 70%. Liver function tests, including ALT, AST, ALP, A/G ratio, albumin, globulin and bilirubin, were also performed in chicks. Overall, keratin-degrading microbial isolates demonstrated strong potential for applications in agriculture and poultry feed production.

1. INTRODUCTION

Poultry meat is widely consumed worldwide due to its high-quality protein content and relatively low-fat levels. The demand for poultry meat and its production have increased substantially, rising from 9 to 120 trillion tons between 1961 and 2016, and are expected to continue increasing in the future, according to reports by the Food and Agriculture Organization of the United Nations (Zul *et al.,* 2020). As a result of this rapid expansion, the poultry industry generates large quantities of feathers as waste by-products. It is estimated that approximately 8.5 billion tons of feathers are produced annually worldwide (Kumawat *et al.,* 2018).

These feathers contribute millions of tons of waste each year, and their disposal poses significant environmental challenges due to the sheer volume generated (Freeman *et al.,* 2009). A single bird produces approximately 125 g of feathers, and nearly five million tons of feather waste are generated weekly due to the production of about 400 million chickens worldwide (Kumar *et al.,* 2017). In the near future, the accumulation of millions of tons of chicken feathers may pose serious risks to human life, as improper disposal contributes to ecological and environmental contamination (Reddy *et al.,* 2021). Poultry manufacturing has significant adverse effects on the environment, leading to land, water, and air pollution, and contributing to the greenhouse effect through the release of CO_2 and N_2O. Nitrous oxide (N_2O)

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emissions primarily result from the extensive use of nitrogen-rich fertilizers, while carbon dioxide (CO_2) is produced during fertilizer manufacturing processes (Tongwane *et al.,* 2016). Additionally, poultry production negatively impacts drinking water quality due to nitrate leaching into groundwater. It has been reported that the poultry sector contributes approximately 18 million tons of CO_2 emissions annually (Ramirez *et al.,* 2006). Rapid microbial decomposition of feather waste leads to the release of noxious gases, including ammonia (NH_3) and hydrogen sulfide (H_2S). Incineration remains the most common disposal method; however, this practice represents a significant loss of valuable resources, as feathers contain approximately 90% protein, 4% sulfur, 50% organic carbon, and around 15% nitrogen (Bohacz *et al.,* 2020).

Alpha-keratin is found in the epithelial layer of tissues in all vertebrates and animals, particularly in structures such as horns, wool, hair, hooves, and nails (Greenwold *et al.,* 2014; Shah *et al.,* 2019). The alpha-helical structure of keratin makes it highly resistant to microbial degradation, thereby posing a significant ecological challenge. Keratin exhibits exceptional strength, durability, and resilience and is largely insoluble in nature (Tongwane *et al.,* 2016). Hydrophobic amino acids, including phenylalanine, isoleucine, methionine, alanine, and valine, are major constituents of keratin. Structurally, alpha-keratin is composed of two sub-filamentous proteins: Type I, which is acidic and has a molecular weight of 40–50 kDa, and Type II, which is basic or neutral with a molecular weight ranging from 50–70 kDa (Jacob *et al.,* 2018).

Beta-keratin, with a molecular weight of 10–14 kDa, is primarily found in nails, shells, claws, and bird beaks. This form of keratin contains a high proportion of cysteine residues, which form disulfide bonds that confer rigidity and resistance to oxidative degradation (Reddy *et al*., 2021). In mature feathers, beta-keratin constitutes approximately 80–90% of the total protein content. The smooth and rigid structure of keratin is largely attributed to its sulfur content, which accounts for nearly 3% of feathers, wool, nails, and horns (Ramakrishnan *et al*., 2018).

Feathers of chickens degraded through physical and chemical methods have several limitations, as these approaches require large amounts of energy during processing and may damage the nutritional quality of the final products (Holker *et al.,* 2016). In recent years, various biotechnological approaches have been explored to reduce keratin waste. Microbial degradation has emerged as an eco-friendly and sustainable alternative, as it preserves the structural integrity of valuable products while minimizing environmental impact (Fang *et al*., 2017). Consequently, current research on biodegradation focuses on the screening and identification of keratin-degrading microorganisms, particularly bacteria and fungi (Elhoul *et al*., 2016). Microbial methods used to produce digestible feather meal are considered environmentally safe and cost-effective, as they operate under mild reaction conditions (Gupta and Ramnani, 2006). Bioconversion of keratin residues not only offers an effective waste management strategy but also enables the production of value-added products (Pasupuleti *et al*., 2008). Therefore, the present study was designed to isolate keratinolytic bacteria from soil and feathers of *Gallus gallus domesticus* (chicken) and to evaluate feather degradation for keratin production, with subsequent application in poultry feed and as a biofertilizer.

MATERIALS AND METHODS

Samples Collection and Feather Powder Preparation

Chicken (*Gallus gallus domesticus*) feathers were collected from a local chicken shop and poultry farm soil was collected from private poultry farm in Doogaje village, Lahore on 18 June 2021. Samples were transported in plastic bag. Feather powder was prepared following the method described by Agrahari and Wadhwa (2016). The powder yield from feathers was calculated using the following formula:

$$\text{Yield} = \text{amount of feather powder} / \text{amount of feathers} \times 100$$

Isolation, characterization, and Screening of Bacterial Isolates from Chicken (*Gallus gallus domesticus*) Feather

Bacterial isolation from poultry farm soil was carried out using a modified method originally described by Martinus (1901), as reported by Kumar *et al*. (2016). The twelve bacterial isolates obtained were cultured on minimal growth medium and incubated at 37 °C for 24 h. Subsequently, the isolates were characterized

based on cultural, morphological, and biochemical properties following the procedures described by

Cappuccino and Sherman (2005). Proteolytic activity was confirmed by streaking purified bacterial isolates onto skim milk agar. Based on the size of the clear (hollow) zones formed, twelve bacterial isolates capable of degrading *Gallus gallus domesticus* feathers were selected.

Effect of different pHs and temperature on bacterial growth and feathers degradation

The effect of different pH levels (5, 7 and 9) and temperatures (25℃, 37℃ and 45℃) on bacterial cell density and feather degradation were determined by measuring optical density at 600 nm and 0.5 nm, respectively, using a spectrophotometer.

Degradation rate of *Gallus gallus domesticus* isolates on pigeon feathers

To evaluate the efficacy of bacterial isolates in feather degradation, pigeon feathers were used in addition to chicken feathers. For this purpose, the isolates were independently incubated in Luria-Bertani (LB) broth at 37°C for 24 h. After incubation, the optical density was adjusted to 0.5 nm. Twelve test tube with 10 ml of fresh keratin medium with feather as the sole carbon source were prepared.

Image analysis using stereomicroscope and Protein Estimation

Feather degradation of *Gallus gallus domesticus* was visually examined after 4 weeks using a stereomicroscope. The protein content of degraded feathers was determined following the method of Lowry *et al*., (1951). For this analysis, cultured flasks containing feathers waste (keratin) were harvested by centrifugation at 13000 rpm for 5 min. After centrifugation, the tubes were placed in a freezer for 2 min, and the pellet and supernatant were separated. The pellet was resuspended in 30 µL of normal saline and further diluted with phosphate buffer at a ratio of 1:4 in Eppendorf tubes. The samples were centrifuged again at 14,000 rpm for 10 min. Subsequently, 400 µL of the supernatant was transferred into test tubes, and 2 mL of Lowry's alkaline reagent was added and incubated at room temperature for 15 min. This was followed by the addition of 0.2 mL of Folin–Ciocalteu reagent, and the absorbance was measured at 750 nm using a spectrophotometer. Protein concentrations were calculated using a standard curve prepared with bovine serum albumin (BSA) at concentrations ranging from 1 to 10 µg/mL.

Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR spectrum of the estimated keratin hydrolysate was analyzed to determine its structural characteristics and compared with spectrum obtained from native keratin. Extracted sample and keratin hydrolysate were sent to a private Laboratory in Lahore, Pakistan and the result were obtained and noted.

Keratin as poultry feeds, Liver function test and its Histopathology

Keratin was also used as feed according to procedure of Fisinin *et al*. (2019) with slight variations. The Feed was prepared in 50% w/v ratio (regular feed = 200g + keratin hydrolysate = 400ml). The feed was soaked in keratin hydrolysate and then air dried. Poultry chicken were used and fed with keratin feed. For this poultry chicken were taken and divided into two groups. One group was labelled as control and other as experimental. A 5-day old chicken were used for each set. Control was fed the regular poultry feed although experimental set was fed with the feed that was soaked in keratin hydrolysate. The initial body weight and body weight after 20 days were recorded. After 30th day of experimentation blood sample from each bird was taken and further analyzed for the liver function test (Bilirubin (BILI), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein (TP), Albumin (ALB), Globulin (GLOB), A/G (albumin/globulin)) of chicks was performed after 30 days. Blood sample of chicks were sent to a private laboratory in Lahore, Pakistan and the result were obtained. Liver was separated after dissection of chicks and Histopathological analysis of liver were performed. For slides preparation chick's liver sample were sent to a private laboratory in Lahore, Pakistan and the result were noted.

Plant microbial interaction (PMI) and keratin as fertilizer

For this selected isolate were inoculated in autoclaved L-broth and incubated for 24 hours at temperature

37°C in incubator (non-shaking). After a day the pellet was harvested through centrifugation for 5 min at 5000 rpm. The supernatant was discarded and the pellet was suspended in autoclaved normal saline. The seeds of fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*) and spinach (*Spinacia oleracea*) were sterilized with $HgCl_2$ for 2 minutes. The sterilized seeds were soaked in different isolate pellets with suspended saline for 20 minutes. The seeds were placed in autoclaved wet filter paper petri dishes. Later, plates were placed in dark for 3 days till germination. After germination the root length, shoot length, dry weight and fresh weight of seedling were noted. The seedling was transferred into water according to Kshetri *et al*. (2018) with slight modification in the process of hydroponic dilution preparation. After 14 days the root length, shoot length, dry weight and fresh weight of seedling were also noted.

Identification of bacterial isolates

The 16S rRNA sequencing of SHC12 was done. SHC12 isolates were sent to a private Laboratory in Lahore, Pakistan and the result were recorded.

Statistical analysis

The statistical analysis was done by Standard deviation, mean, two-way ANOVA (p value ≤ 0.05) and least Significance Difference (LSD) using MS Word Excel 2016.

3. RESULTS

Preparation of feather powder

The color of *Gallus gallus domesticus* feather's powder was white color with the yield of 55 %. While the powder of *Columba livia domestica* feathers was black, brown in color with the yield of 46 % (Fig. 1).

Qualitative analysis for keratinolytic activity using skim milk assay

Purification of selected bacterial isolates from enrichment culture was completed by streaking on skim milk agar petri plates for proteolytic activity. From 30 different bacterial colonies, 12 isolates showed positive results in the form of clear zones and further purified by streaking. Isolates from *Gallus gallus domesticus* feathers that showed clear zones were SMC1, SMC2, SMC3, SMC4, SMC5, SMC6, SMC7, SMC8, SMC9, SMC10, SMC11 and SMC12 on skim agar plate (Fig. 2).

Biochemical Characterization of bacterial isolates

Biochemical characterization of isolates was shown in Table 1.

Effect of varying temperature and pHs on bacterial growth

Effect of different temperature on bacterial growth showed a general trend of increased cell densities from temperature 37℃and 45℃ as compared to 25℃ for bacterial isolates SHC1, SHC5, SHC6 and SHC8. Temperature 45℃ was significantly capable for improving cell densities of bacterial isolates SHC2, SHC3, SHC4, SHC7, SHC9, SHC10, SHC11 and SHC12 when compared to temperature 25℃ and 37℃ (Fig. 3). The effect of temperature with different isolates was further analyzed statistically by two-way ANOVA and a significant difference was noticed in cell densities ($p<0.05$) at varied temperature treatments.

Effect of different pH on bacterial growth showed a general trend of increased cell densities from pH 7.0 and pH 9.0 as compared to pH 5.0 for bacterial isolates. pH 9.0 and 7.0 were significantly capable of improving cell densities of isolates SMC4 and SMC12 when compared to pH 5.0 (Fig. 4). The effect of pH with different isolates was further analyzed statistically by two-way ANOVA and a significant difference was noticed in cell densities ($p<0.05$) at varied pHs treatment.

Effect of varying temperature and pH on degradation of feathers

To examine the effect of varying pH (5, 7 and 9) and temperatures (25°C, 37°C and 50°C) on keratin degradation, isolates were incubated at respective culture conditions for keratinolytic activity in the presences of bacterial inoculum added at optical density (0.5 nm). Moreover, bacterial inoculation resulted in noticeable degradation because degradation time was decreased by 15 days as compared to control. Higher temperature as decreased the time from 4 weeks to almost 2 weeks (Fig. 5). The amount of keratin per gram feather powder weight was maximum at 45°C and pH 9.0 (Fig. 6) as compared to rest of culture conditions. In general, high temperature and

high pH favored keratin production in the presence of bacterial consortia.

Degradation rate of isolates on pigeon *(Columba livia domestica)* feathers

The degradation rate of Gallus *gallus domesticus* isolates on pigeon feathers was also studied. Results demonstrated the degradation rate of all isolates (SHC1, SHC2, SHC3, SHC4, SHC5, SHC6, SHC7, SHC8, SHC9, SHC10, SHC11 and SHC12) were more pronounced on pigeon feather as compared to control (Fig. 7).

Stereo Micrographs of Keratin

Stereomicroscope was used to notice the pattern of feather degradation during the study (Fig. 8). It was noticed the feathers were completely degraded into white powdery mass till 4th week.

Growth on Minimal Agar

The isolates were streaked on Minimal agar plates and growth was noticed after 24 hours incubation at 37°C.

Protein analysis of extracted keratin

The protein study of the estimated keratin from different bird's feathers *Columba livia domestica* and *Gallus gallus domesticus* feathers were noticed as 7.818 mg/ml and 3.949 mg/ ml respectively.

Fourier Transform Infrared (FTIR) spectroscopy analysis

Fourier transform infrared (FTIR) spectroscopy was employed to identify the functional groups of chicken and pigeon keratin hydrolysates based on their characteristic absorption peaks, as shown in Fig. 9 and Fig. 10.

Keratin as poultry feeds

Result demonstrated that keratin as feed provided effective results. The weight of experimental setup of birds were more as compare to control setup. The maximum weight increase percentage was observed for chick 2 in experimental setup which was 80 % and in case of control setup the greatest weight increase percentage was observed for chick 3 which was 65%. initial weight was 76g and final weight was 94 g of chick 2 in experimental setup. Chick 2 of control setup had its initial weight 64g and final weight was 88g (Table 2).

Liver function test of chicks

Results demonstrated that the level of bilirubin was 0.8 mg/dL for all six experimental and control chicks. The average ALT level for control chicks was 29.6 U/L, whereas for experimental chicks it was 16.6 U/L. The average AST level for control chicks was 214.6 U/L, compared to 187.3 U/L in the experimental group. ALP levels averaged 15,581 U/L in control chicks and 3,777.3 U/L in experimental chicks. Total protein was higher in the experimental group (4.9 g/dL) compared to controls (4.2 g/dL). Albumin levels were 1.56 g/dL in controls and 1.8 g/dL in experimental chicks. Globulin levels were 2.63 g/dL for control chicks and 13.9 g/dL for experimental chicks. Consequently, the albumin/globulin (A/G) ratio increased from 0.59 in controls to 1.47 in the experimental group (Table 3).

Histopathological test of chick's liver

Liver tissue slides were examined under a light microscope at 100× magnification. The observations indicated that keratin supplementation in feed caused no detrimental effects on liver tissue, as hepatocytes appeared healthy. Both the control and experimental groups showed intact and normal cellular structures of hepatocytes (Fig. 11).

Plant Microbial Interaction (PMI) and Keratin as Fertilizer

Plant–microbial interaction (PMI) experiments were conducted to evaluate the efficiency of bacterial isolates on the germination and growth of fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*), and spinach (*Spinacia oleracea*). The results showed a significant increase ($p < 0.05$) in germination and seedling growth compared to the control. Among the isolates, SHC4 and SHC12 demonstrated the highest efficacy ($p < 0.05$) for promoting germination and growth across all three-plant species (Table 4). Furthermore, keratin hydrolysate used as a fertilizer significantly enhanced plant growth, particularly at 100× and 70× dilutions, improving root and shoot lengths compared to other treatments and controls (Table 5).

Identification of bacterial isolate

Genetic identification and phylogenetic relationship of the bacterial isolate were performed using 16S rRNA gene sequencing. The strain was identified based on

sequence homology using BLAST analysis, and the 16S rRNA gene sequence (1,497 bp) was submitted to NCBI Genbank. The sequence of isolate SHC12 showed 100% similarity to Pseudomonas strain DSM 5425 and was designated as Pseudomonas sp. strain SHC12 785F, with the assigned GenBank accession number OM952169 (Fig. 12).

4. DISCUSSION

Proteins play a fundamental role in maintaining body structure and physiological functions. Poultry feathers represent a valuable protein source due to their high content of amino acids such as glycine, alanine, serine, cysteine, valine, and lysine, although methionine and tryptophan are present in relatively lower amounts. In the present study, bacterial isolates demonstrated efficient feather degradation over a broad range of temperatures and pH conditions. This observation is consistent with previous findings indicating that keratinolytic bacteria often exhibit optimal keratin degradation under alkaline and elevated temperature conditions, reflecting their adaptability to harsh environments (Călin *et al*., 2017). Protein analysis revealed that *Columba livia domestica* feathers contained a higher protein content (7.994 mg/100 ml) compared to *Gallus gallus domesticus* feathers was 3.949 mg/100 ml. These findings are in agreement with Martelli *et al*. (2006), who reported that keratin extraction from feathers can yield high protein concentrations, reaching up to 12 g/100 mL under optimized conditions.

Fourier Transform Infrared spectroscopy was employed to identify the functional groups present in the feather keratin hydrolysate. Broad absorption bands observed at 3513–3413 cm^{-1} corresponded to O–H stretching vibrations, while the peak at 2976 cm^{-1} was attributed to C–H stretching associated with the Amide III region. The absorption band at 1634 cm^{-1} represented C=O stretching characteristic of the Amide I group, whereas the peak at 1449 cm^{-1} corresponded to N–H bending of the Amide II group. Peaks at 1084, 1043, and 878 cm^{-1} were associated with S=O stretching vibrations, indicating the presence of sulfur-containing amino acids such as cysteine. The absence of major peak shifts suggested that no significant alterations occurred in the chemical structure of keratin following hydrolysis. These results are consistent with FTIR analyses reported by Ma *et al*. (2016), Sharma *et al*. (2017), and Badrulzaman *et al*. (2021).

The keratin hydrolysate produced by bacterial isolates was further evaluated for its effect on seed germination and early plant growth under hydroponic conditions using fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum)* and spinach (*Spinacia oleracea*) seeds. High concentration of feather hydrolysate was inhibited seeds germination of plants. Our results demonstrated that 100x dilution was highly significant (p <0.05) for seeds germination. Zul *et al.,* (2020) was analyzed feather organic fertilizer on the growth of spinach plant in soil. After 30 days the shoot length of spinach plant (16.5 cm/plant) was noted at feather organic fertilizer application although the shortest (14.0cm/plant) was in control.

Çakmakçı *et al*. (2007) was examined the effect of different *Bacillus* sp. on the growth of wheat and spinach plant. *Bacillus* sp. improved wheat shoots fresh weight by 16.2%–53.8% and spinach shoots fresh weight by 2.2%–53.4% as compared to control control. *Bacillus* sp. improved plant height by 2.2%–24.6% and 1.9%–36.8% in wheat and spinach, individually. In plant microbial interaction (PMI) our results demonstrated that isolates SHC4 and SHC12 was vastly significant (p <0.05) for fenugreek, coriander and spinach seeds germination and plant growth. Popko *et al*. (2015) evaluated the efficacy use of NKSMg fertilizer on the germination of rape seed. This study was designed at the assessment of the effects of the feed additives founded on keratin comprising wastes on the body weight and liver function of chicks. Control was fed the regular poultry feed although experimental set was fed keratin hydrolysate feed. The maximum weight increase percentage was observed in experimental setup which was 80% and in case of control setup the highest weight increase percentage was observed 65 %. Fisinin *et al*. (2019) reported that keratin hydrolysate increases the body weight of chickens up to 9-10 % as compare to fishmeal. Torok *et al*. (2011) monitor strategies to advance feed efficiency and feed design for best gut health. Keratin on the basis of amino acid content used as feed (McKerns and Rittersporn, 1958).

Volik *et al*. (2020) considered about biochemical properties of biologically active feed extracts found from raw materials from poultry waste comprising keratin and collagen. Results demonstrated that increase in live weight of chicken to be 8.6 % ($p < 0.001$) as compare to control.

ALT and AST enzyme is originated in maximum quantity in liver and is used to distinguish acute liver failures as the enzyme is out into the serum proximately after a hepatocellular injury (Senanayake *et al*., 2015). But in this study the level of ALT for control chick on average was 29.6 U/L and for experimental chick was 16.6 U/L. AST level for control chicks on average 214.6 U/L and for experimental chick was 187.3 U/L. Aalkaline phosphate is an enzyme which is associated by the biliary tract. It is as well originated in bone and the placenta. Renal or intestinal injury can reason the ALP to increase. Once it is existing in bulky quantities, it may show liver or bone disease or a tumour (Manan *et al*., 2012). Our study indicates the ALP level for control chick on average was 15581 U/L and for experimental chick was 3777.3 U/L. Noxious injury of the liver of chickens was attended via an important increase of bilirubin and enzymes of transamination in the serum (Reznichenko *et al*., 2017). Results demonstrated that level of Bilirubin is 0.8 mg/dl for all 6 experiment and control chicks. Zaefarian *et al*. (2019) stated that the secretion rate in liver of chicken is 0.9 µg/kg/min. Total protein was higher for experimental setup as compare to control setup. The total protein level for experimental chick on average was 4.9 g/dl while the control had 4.2 g/dl. The Albumin was increased for experimental setup as compare to control setup. The Albumin level for experimental chick on average was 1.8 g/dl while the control had 1.56 g/dl. The globulin was increased for experimental setup as compare to control setup. The globulin level for experimental chick on average was 13.9 g/dl while the control had 2.63 g/dl. Greenacre and Morishita (2021) penned in their book under the title of Blood chemistry for chicken the standard 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. The ratio of albumin/globulin was increased for experimental chick as compare to control chick. The albumin/globulin ratio for experimental chick on average was 1.47 while the control had 0.59. Tóthová *et al*. (2019) reported the albumin/globulin ratio for chicken from day 4 to day 46 to be 0.59-0.85. Café *et al*. (2012) described the albumin/globulin ratio for chicken from day 14 to day 42 to be 1.50-1.72.

5. CONCLUSION

The twelve bacterial isolates obtained from poultry farm soil demonstrated strong chicken feather degradation ability and efficient keratin production. Among them, isolates SHC4 and SHC12 exhibited the highest degradation efficiency, achieving complete degradation of chicken feathers within 20 days at 45 °C and pH 9.0. These isolates were also capable of degrading feathers from other bird species, such as pigeon, at 37 °C. Protein analysis revealed that chicken feathers contained lower protein content (3.9 mg/mL) compared to pigeon feathers (7.818 mg/mL). The keratin hydrolysate significantly enhanced seed germination and growth of fenugreek, coriander, and spinach, with a maximum plant biomass increase of 70.7% observed following keratin supplementation. Based on molecular identification, isolates SHC4 and SHC12 were identified as Pseudomonas spp. and demonstrated substantial bioremediation potential for the recycling of keratinous waste, particularly poultry feathers. The findings highlight their promising application as sustainable organic fertilizer and poultry feed additives. Keratin hydrolysates derived from chicken feathers may therefore serve as valuable biomaterials for agricultural and environmental applications.

6. CONFLICT OF INTEREST

The authors have declared no conflict of interest regarding the publication of this article.

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Table 1. Biochemical characterization of isolates was shown in Table 1.

Gram negative bacteria	Gram positive bacteria	Non-Spore forming and negative indole production test	Capsulated	Non-capsulated	Positive methyl red, catalase, urease, citrate, motility, oxidase, voges-Proskeur and DNase test	Negative DNase test	Negative oxidase test	Negative voges-Proskeur test
SMC1, SMC3, SMC4, SMC5, SMC6, SMC9, SMC10 and SMC12	SMC2, SMC7, SMC8 and SMC11	SMC1, SMC2, SMC3, SMC4, SMC5, SMC6, SMC7, SMC8, SMC9, SMC10, SMC11 and SMC12	SMC1, SMC4, SMC5, SMC6, SMC7, SMC9 and SMC10	SMC2, SMC3, SMC8, SMC11 and SMC12	SMC1, SMC2, SMC3, SMC4, SMC5, SMC6, SMC7, SMC8, SMC9, SMC10, SMC11 and SMC12	SMC1, SMC2 and SMC11	SMC1 and SMC4	SMC11

Table 2. Effect of feather keratin hydrolysate on chicken weight

Chicken	Control set-up (Without keratin feed)			Experimental set-up (With keratin feed)		
	Initial weight (g)	Final weight (g)	Weight increase %	Initial weight (g)	Final weight (g)	Weight increase %
Chick 1	90 ± 1.41	148 ± 2.82	60 ± 2.12	64 ± 1.40	88 ± 1.41	72 ± 2.82
Chick 2	76 ± 3.36	156 ± 1.41	48 ± 1.41	76 ± 1.41	94 ± 2.82	80 ± 2.12
Chick 3	62 ± 2.21	94 ± 3.24	65 ± 0.70	77 ± 0.70	100 ± 5 .65	77 ± 0.71

Table 3. Liver function test of chicks

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	1.0 ± 0.17	13.9 ± 0.15	1.47 ± 0.01
Bilirubin (BILI), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein (TP), Albumin (ALB), Globulin (GLOB), A/G (albumin/globulin).								

Table 4. Effect of feather protein hydrolysate on fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum)* and spinach (*Spinacia oleracea*) plants.

Plants	Treatment	Soluble protein content (mg ml^{-1})	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
Fenugreek	**30x**	0.1	2.8 ± 0.14	5.3 ± 0.11	0.0214 ± 0.007	0.0060 ± 0.003
	50x	0.06	3.0 ± 0.21	5.6 ± 0.07	0.0245 ±0.005	0.0063 ± 0.005
	70x	0.04	3.1 ± 0.28	5.7 ± 0.14	0.0270 ±0.002	0.0066 ±0.002
	100x	0.032	3.5 ± 0.56	6.0 ± 0.63	0.0272 ±0.004	0.0018 ±0.001
	Control	0.000	2.4 ± 0.26	4.6 ± 0.56	0.0191 ±0.001	0.0022 ±0.002
Coriander	**30x**	0.1	5.1 ± 0.20	6.4 ± 0.35	0.0532 ±0.003	0.0078 ±0.004
	50x	0.06	5.0 ± 0.49	6.9 ± 0.77	0.0590 ±0.001	0.0086 ±0.001
	70x	0.04	5.4 ± 0.35	7.5 ± 0.56	0.0615 ±0.004	0.0096 ±0.005
	100x	0.032	6.8 ± 0.41	7.6 ± 0.35	0.0690 ±0.003	0.0042 ±0.006
	Control	0.000	4.9 ± 0.35	5.9 ± 0.28	0.0426 ±0.002	0.0031 ±0.003
Spinach	**30x**	0.1	2.9 ± 0.40	6.6 ± 0.42	0.0599 ±0.006	0.0041 ±0.004
	50x	0.06	3.1 ± 0.21	6.8 ± 0.13	0.0601 ±0.008	0.0077 ±0.001
	70x	0.04	3.1 ± 0.28	6.9 ± 0.76	0.0646 ±0.005	0.0079 ±0.007
	100x	0.032	3.3 ±0.12	7.2 ± 0.33	0.0702 ±0.002	0.0052 ±0.004
	Control	0.000	2.6 ± 0.26	4.4 ± 0.21	0.0174 ±0.006	0.0034 ± 0.003
LSD for Fenugreek = 0.39 LSD for coriander = 0.65 LSD for Spinach = 0.75						

Table 5. Effect of plant-microbial interaction on shoot, root length and germination percentage of fenugreek, coriander and spinach plants

Isolates	Fenugreek			Coriander			Spinach		
	Root length (cm)	Shoot length (cm)	Germination percentage	Root length (cm)	Shoot length (cm)	Germination percentage	Root length (cm)	Shoot length (cm)	Germination percentage
SHC1	0.4 ± 0.10	3.1 ± 0.63	73 ± 4.94	0.7 ± 0.42	1.8 ± 0.28	66 ± 5.65	1.6 ± 0.16	3.1 ± 0.14	80 ± 2.12
SHC2	0.6 ± 0.11	3.3 ± 0.56	76 ± 7.07	0.6 ± 0.36	1.9 ± 0.70	96 ± 6.26	1.4 ± 0.26	3.0 ± 0.18	60 ± 2.82
SHC3	0.4 ± 0.07	3.2 ± 0.42	80 ± 2.12	1.7 ± 0.21	2.2 ± 0.21	80 ± 7.07	1.9 ± 0.14	3.3 ± 0.21	66 ± 7.07
SHC4	0.9 ± 0.06	3.7 ± 0.84	86 ± 2.82	1.8 ± 0.07	2.4 ± 0.42	86 ± 6.36	2.3 ± 0.18	3.7 ± 0.27	96 ± 5.56
SHC5	0.6 ± 0.15	3.0 ± 0.14	96 ± 5.65	0.7 ± 0.77	1.9 ± 0.22	66 ± 9.19	2.1 ± 0.11	3.5 ± 0.20	76 ± 3.53
SHC6	0.7 ± 0.05	3.4 ±0.77	76 ± 4.24	1.0 ± 0.42	2.1 ± 0.43	76 ± 2.82	2.0 ± 0.24	3.2 ± 0.12	66 ± 9.19
SHC7	1.0 ± 0.22	3.6 ± 0.98	96 ± 5.65	1.5 ± 0.28	1.6 ± 0.71	80 ± 7.07	1.8 ± 0.34	3.4 ± 0.17	76 ± 4.24
SHC8	0.8 ± 0.17	3.5 ± 0.14	86 ± 6.36	1.7 ± 0.35	1.9 ± 0.84	73 ± 9.19	2.5 ± 0.21	3.5 ± 0.16	70 ± 2.82
SHC9	0.5 ± 0.12	3.1 ± 0.56	73 ± 8.48	1.3 ± 0.22	1.8 ± 0.77	80 ± 7.07	2.1 ± 0.16	3.4 ± 0.10	76 ± 1.41
SHC10	0.4 ± 0.07	3.0 ± 0.35	80 ± 5.65	0.9 ± 0.21	1.5 ± 0.49	73 ± 2.82	1.8 ± 0.11	2.9 ± 0.11	73 ± 6.36
SHC11	0.8 ± 0.17	3.6 ± 0.70	86 ± 6.36	1.5 ± 0.28	2.1 ± 0.56	86 ± 1.41	2.1 ± 0.01	3.6 ± 0.06	80 ± 0.70
SHC12	1.1 ± 0.11	3.9 ± 0.14	96 ± 9.89	1.9 ± 0.14	2.6 ± 0.63	96 ± 5.55	2.8 ± 0.04	3.9 ± 0.24	86 ± 2.12
Control	0.3 ± 0.03	2.1 ± 0.63	66 ± 7.11	0.5 ± 0.27	1.3 ± 0.40	65 ±7.17	0.5 ± 0.12	1.1 ± 0.15	53 ± 4.24

Spinach (LSD for isolates = 0.155; LSD for treatment = 0.397)

Coriander (LSD for isolate = 0.146; LSD for treatment = 0.528)

Fenugreek (LSD for isolates = 0.14; LSD for treatment = 0.38)

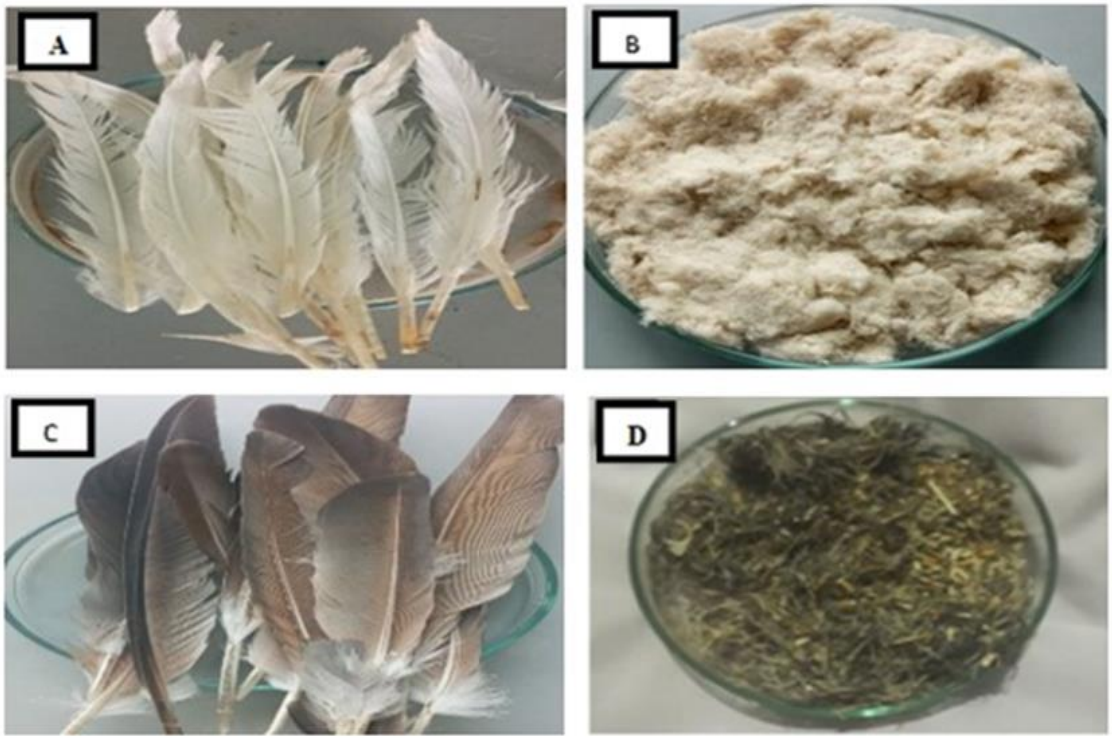

Figure 1. A-B *Gallus gallus domesticus* feathers and powder respectively; C-D *Columba livia domestica* feathers and powder respectively

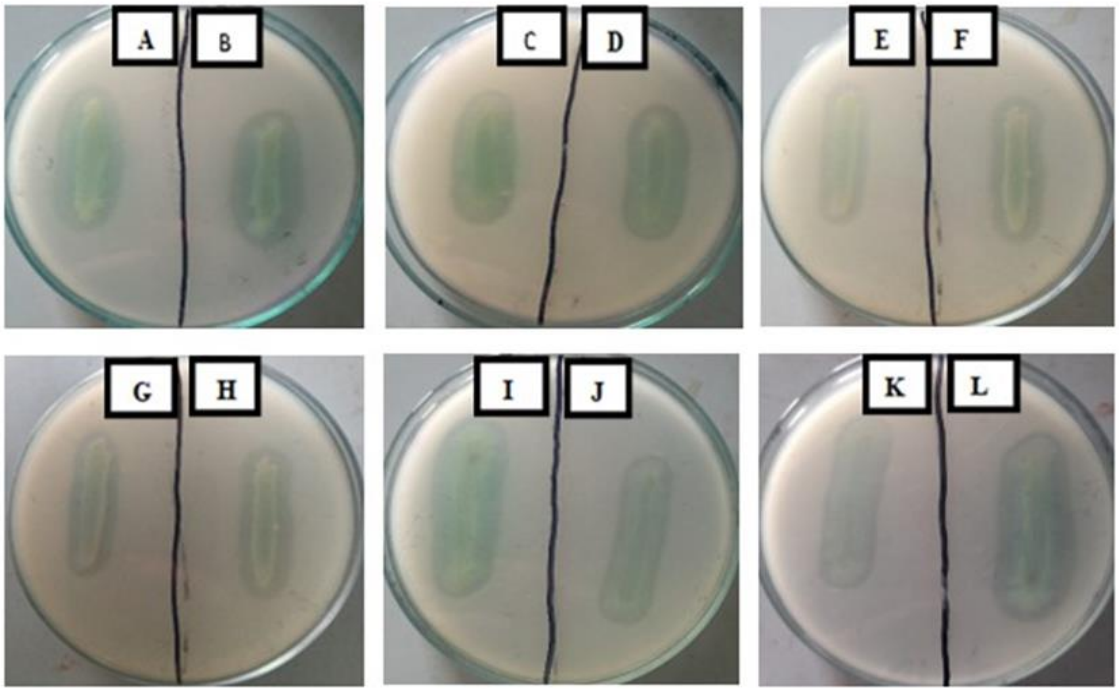

Figure 2. Initial screening of bacterial isolates (A-L= SMC1, SMC2, SMC3. SMC4, SMC5, SMC6, SMC7, SMC8, SMC9, SMC10, SMC11 and SMC12) on the basis of Clear zones on skim milk agar plate.

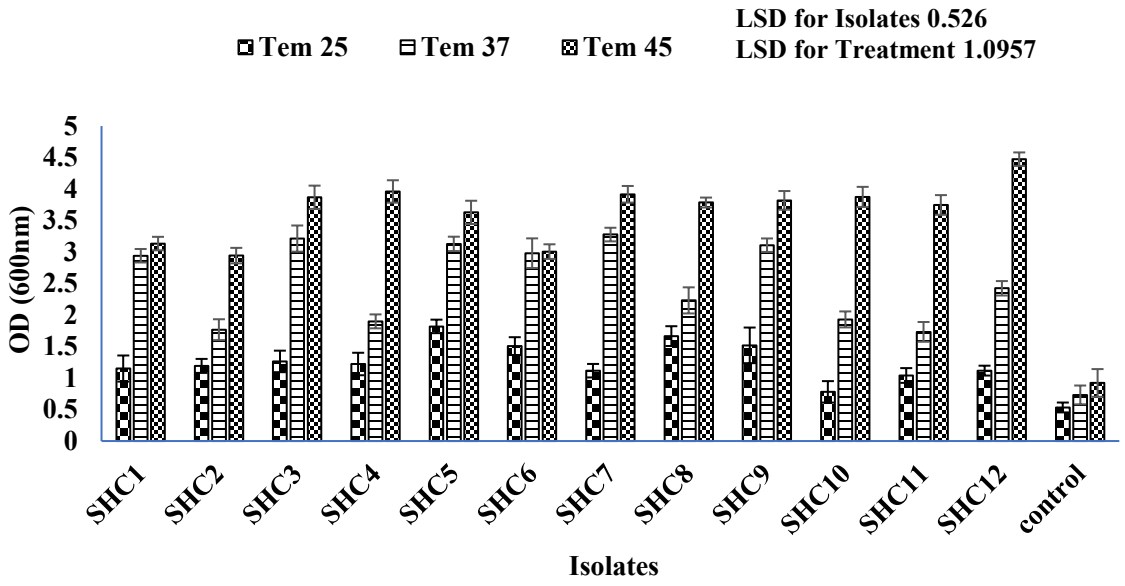

Figure 3. Effect of different temperature on bacterial growth.

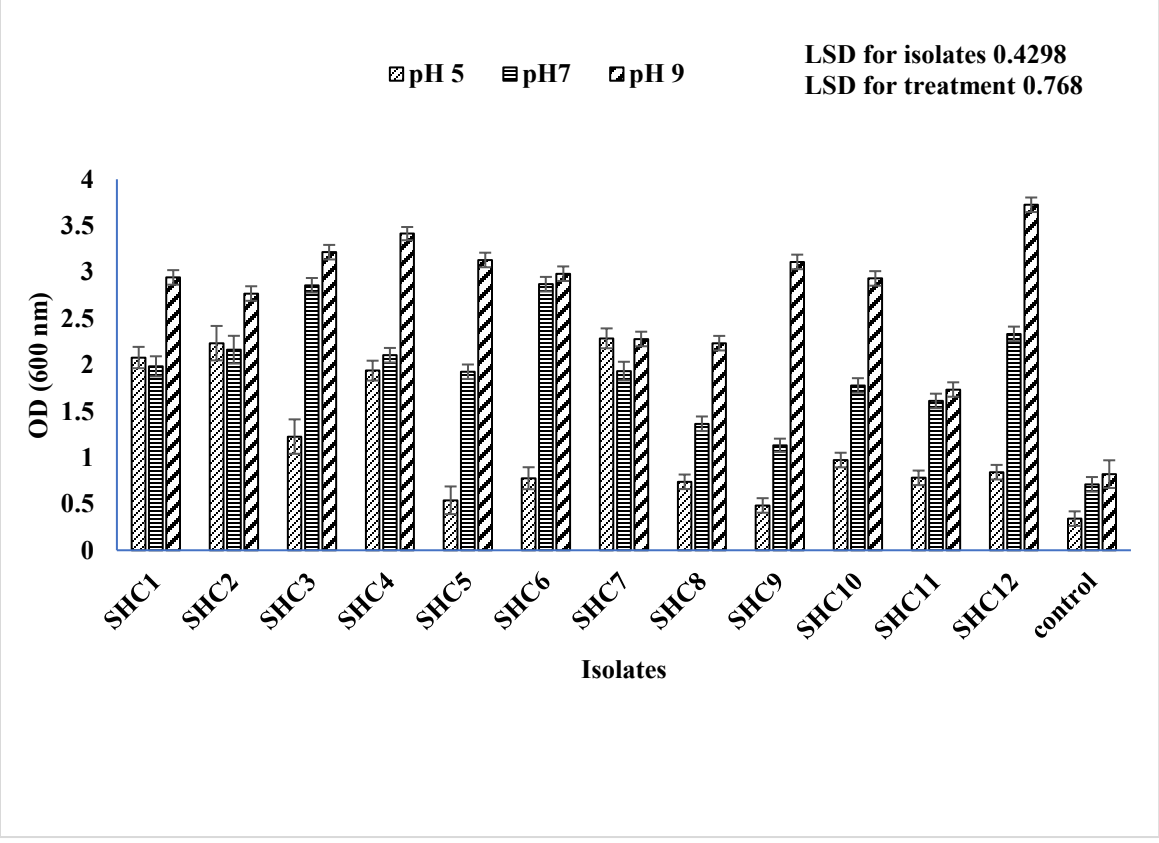

Figure 4. Effect of different pH on bacterial growth.

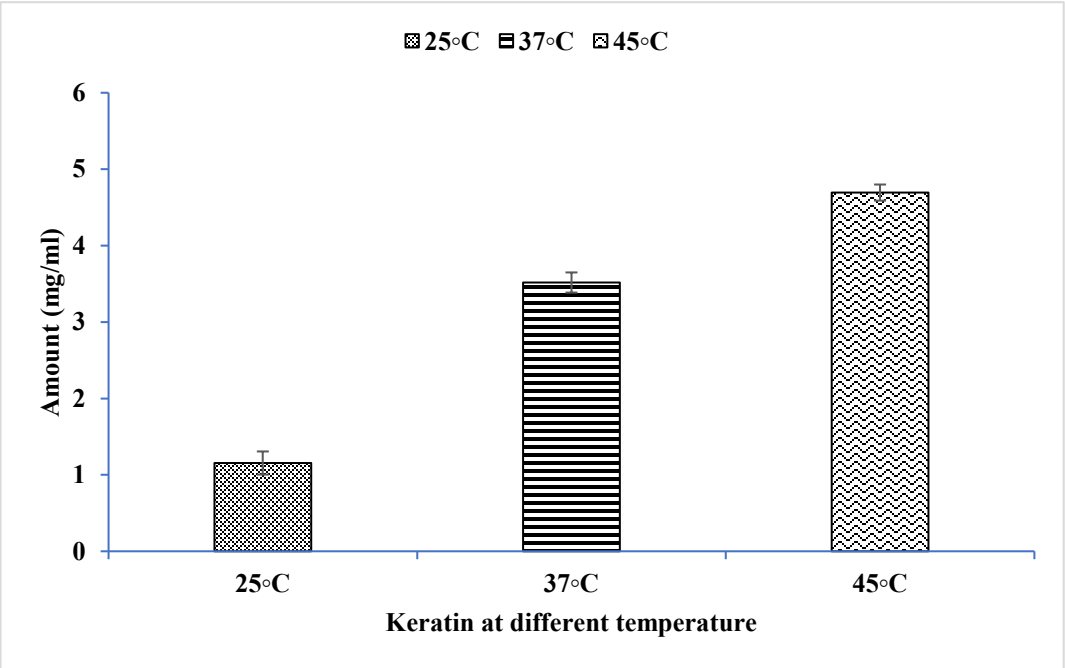

Figure 5. Amount of Keratin obtained at different pH

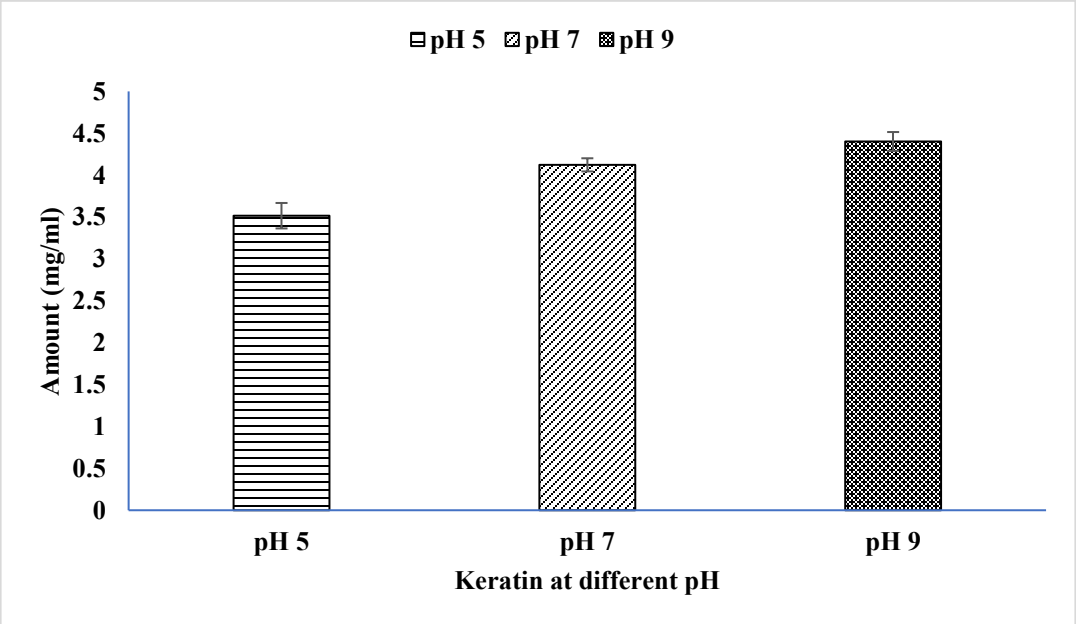

Figure 6. Amount of Keratin obtained at different temperatures.

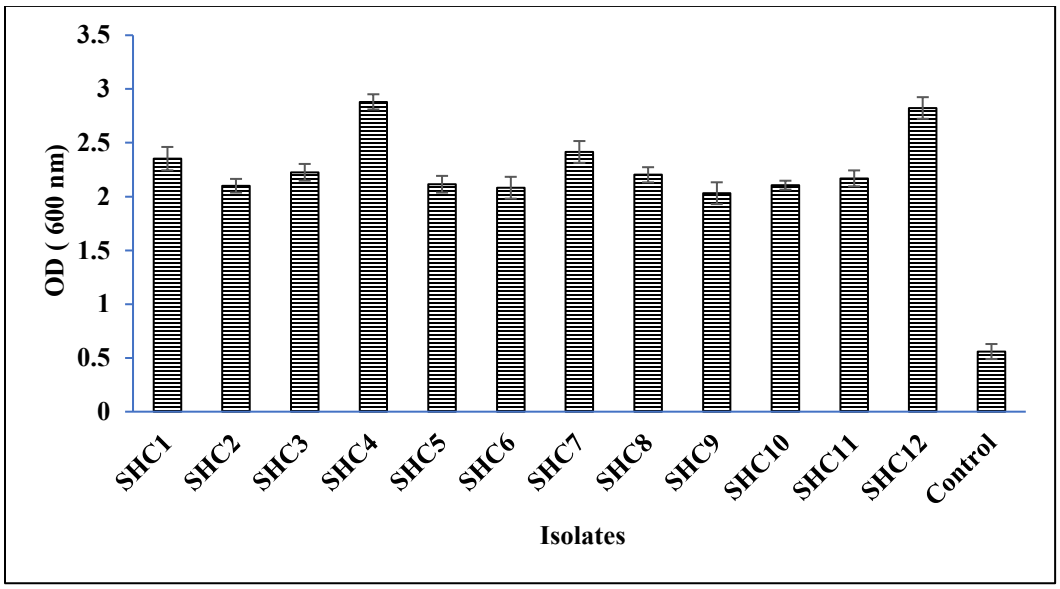

Figure 7. Degradation of pigeon feathers with isolated bacteria's

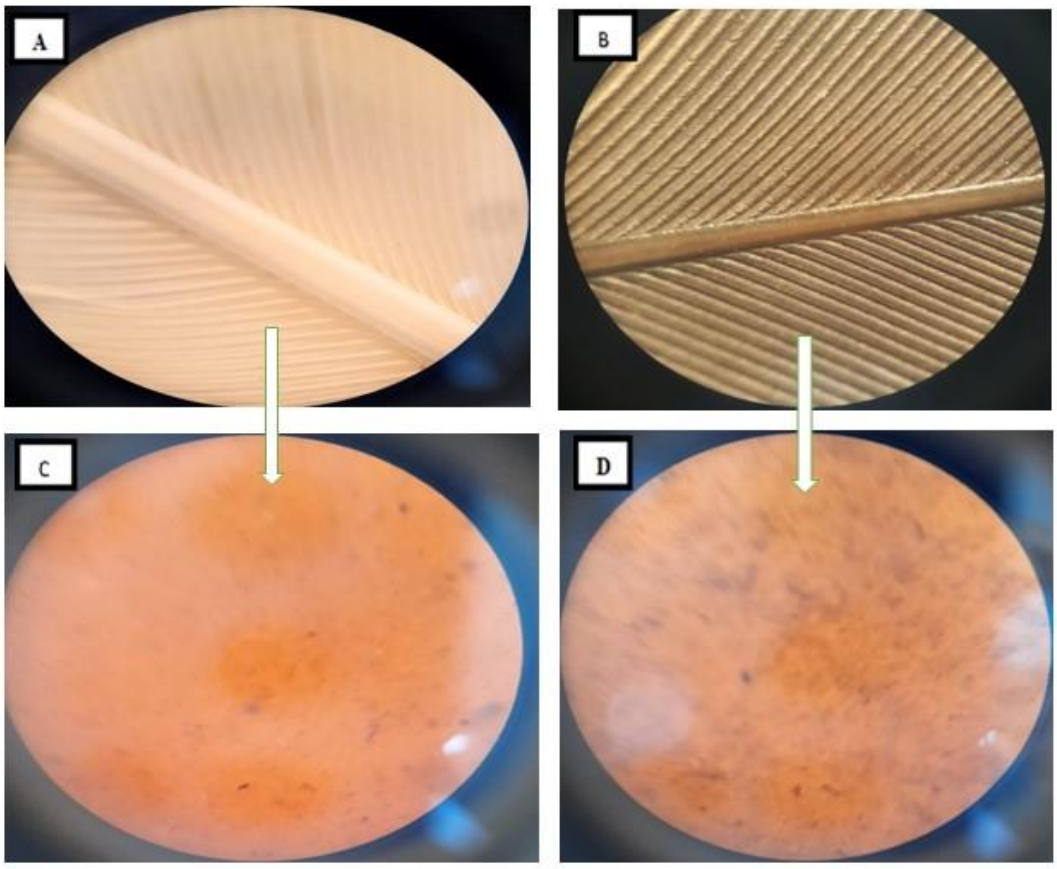

Figure 8. Stereo micrographs of feather degradation; (A = *Gallus gallus domesticus* feather; B = *Columba livia domestica* feather; C = Stereo-micrograph of degradation of *Gallus domesticus* feathers; D = Stereo-micrograph of degradation of *Columba livia domestica* feathers).

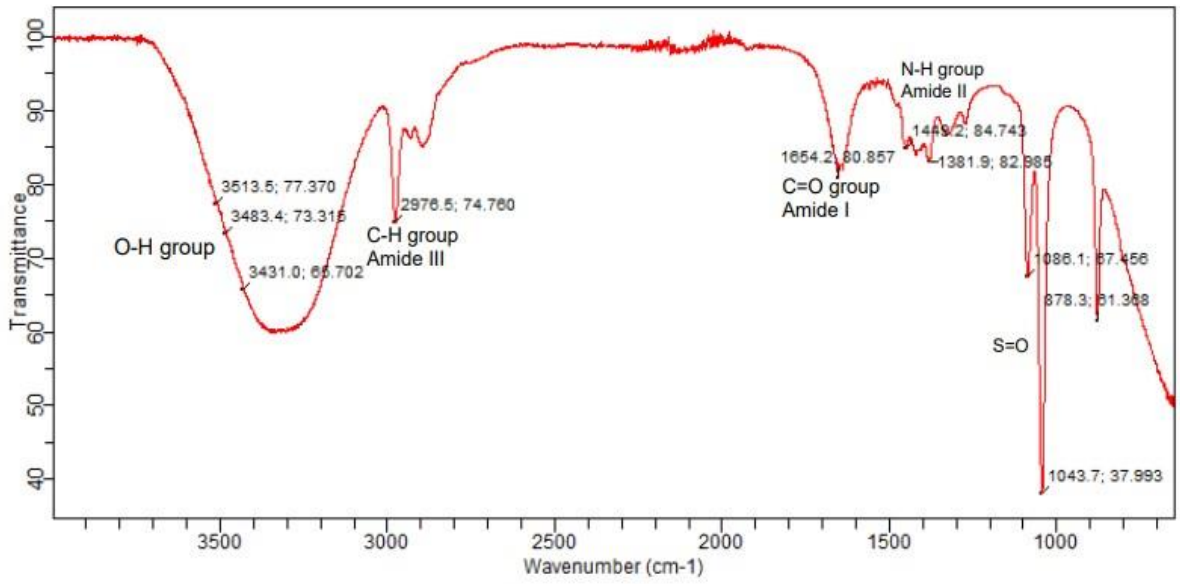

Figure 9. FTIR of chicken (*Gallus gallus domesticus*) keratin hydrolysate

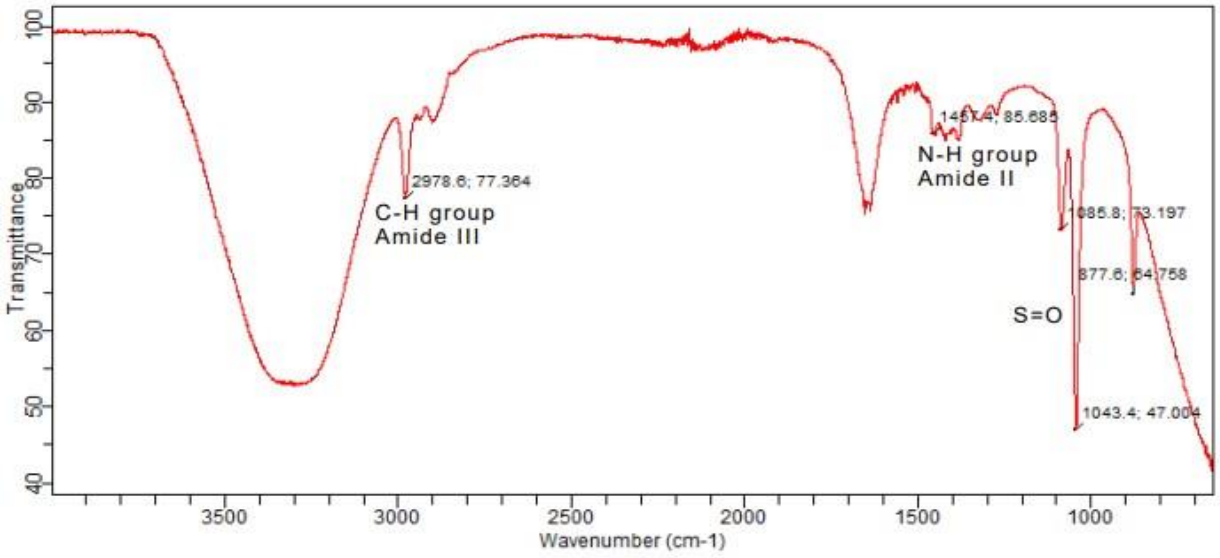

Figure 10. FTIR of pigeon (*Columba livia domestica*) keratin hydrolysate

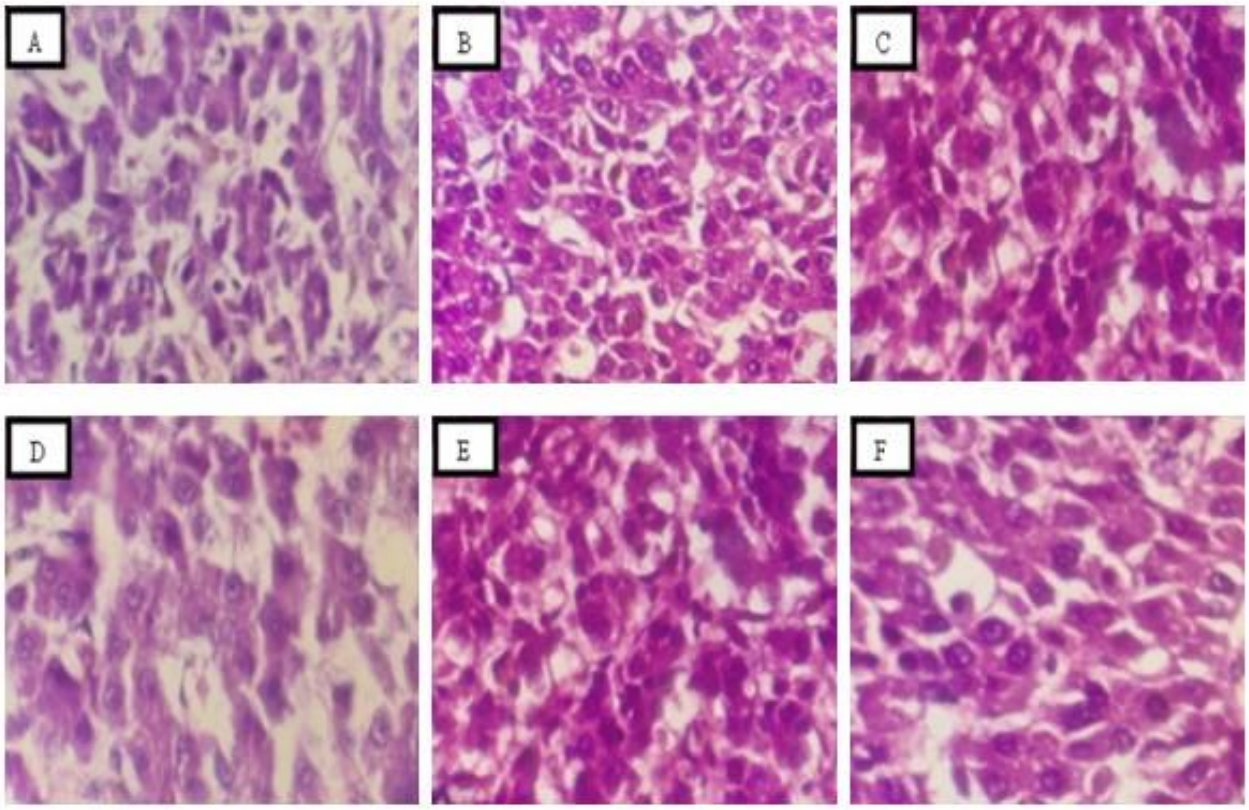

Figure 11. Histopathological test of chick's liver (A-C = histopathology of experiment group, D-F= histopathology of control group).

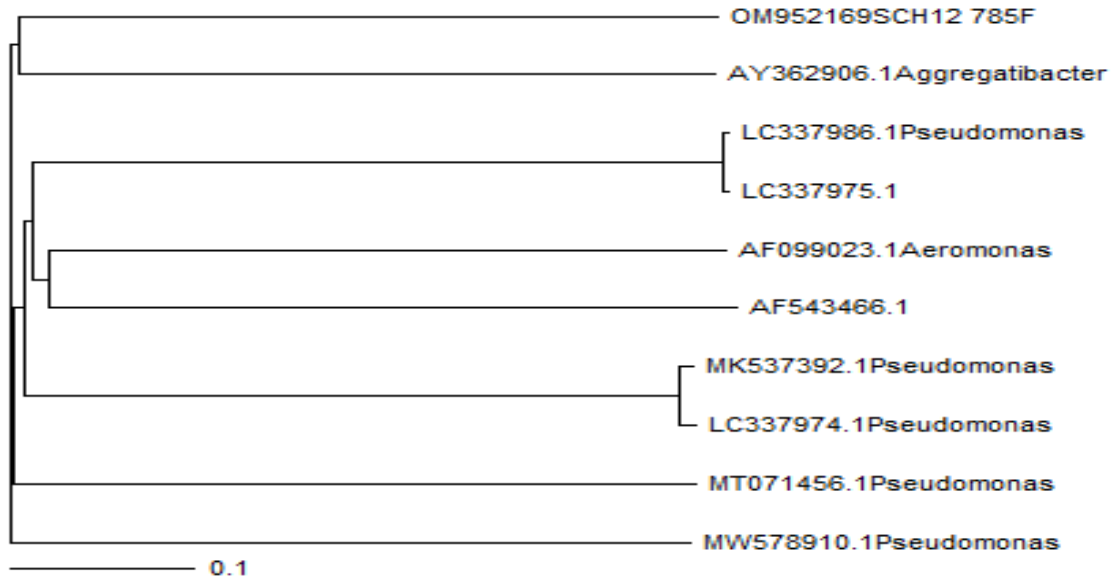

Figure 12. Phylogenetic tree based on 16S rRNA gene partial sequences of bacterial strains using the Neighbor-Joining method with the help of Clustal w software (Saitou and Nei., 1987). Clustering of bacterial cells in the groups, subgroups, sections and subsections shows their common ancestral origin.