

(RESEARCH ARTICLE)



Microbial degradation of Chicken Feather Waste (CFW) by *Paenibacillus koreensis* YC300

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World Journal of Biology Pharmacy and Health Sciences, 2026, 25(02), 245-255

Publication history: Received on 14 May 2025; revised on 13 February 2026; accepted on 16 February 2026

Article DOI: <https://doi.org/10.30574/wjbphs.2026.25.2.0511>

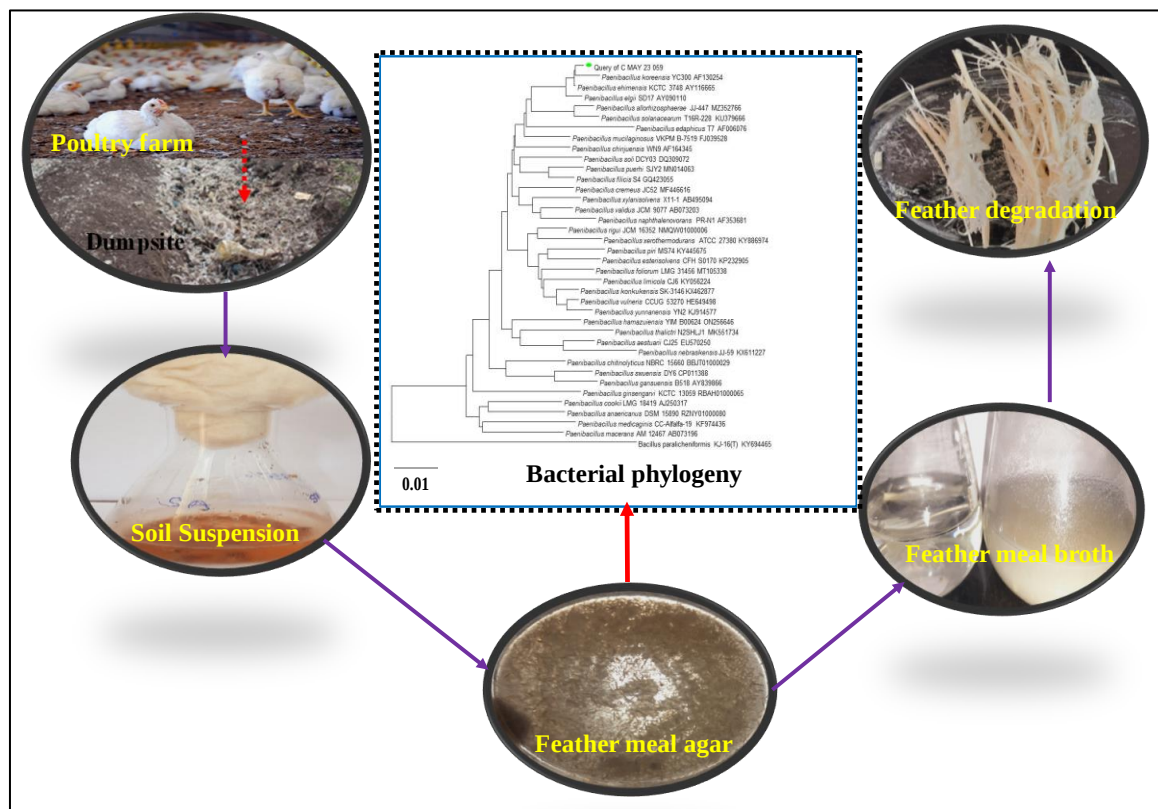
Abstract

Poultry has a prominent role in modern food industry contributing many nutritious products. A major byproduct of the poultry industry is chicken feather waste (CFW) which is rich in keratin and is generated in excessive amounts from poultry farms. The poultry industry thus generates huge quantities of solid CFW resistant to degradation leading to serious environmental pollution. It is possible to harness the capacity of keratinases from natural sources to degrade insoluble keratin present in CFW. The current communication outlines the isolation and screening of CFW-degrading bacteria from poultry soil. The potent keratin degrading bacterium was enriched on keratin-agar medium and identified as *Paenibacillus koreensis* YC300 by 16S rRNA gene and phylogenetic studies.

Keywords: Poultry feather waste; Keratinolytic bacteria; 16S rRNA gene; *Paenibacillus koreensis* YC300

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Graphical abstract



1. Introduction

Poultry farms and industry are progressing rapidly, the development in this area is highly favored since this supply protein rich food and career opportunities to people. The consumption of poultry and its products in the modernized world is increasing nowadays due to their nutritional quality and advancement in food processing and preparation. Along with the benefits from poultry industry to the population, the disadvantage like solid waste management is problematic (Brandelli et.al. 2008⁶ and Godheja et.al. 2014¹⁰). India is considered the fifth largest manufacturer of chicken with 4.2 million tons annually (Singh et. al.2019³⁹), with feathers waste alone constituting nearly 8.5% of the total chicken weight. Broadly, 8.5 billion tons of CFW are generated each year. In the study 2018, India yielded 4.6 million tons of broiler chicken (Prabhavathi et.al. 2018³³), and roughly 350 million tons of feather waste was accumulated (Samuel et.al. 2018³⁶). A large variety keratin-rich waste like feather, wool, and leather are directly dumped, landfilled, or incinerated once a year which leads to loss of resources and also causes environmental pollution (Akhter et.al. 2020¹). As a result, the usage of keratinous waste supply is an immediate issue to be resolved. Several methods are available for the management of feather waste. In common, feathers were boiled at high temperatures and pressure to form feather meal which is used as a component in animal feeds. The feather meal production methods involve two processes; hydrothermal treatments and microbial degradation. The hydrothermal method is costly and generates denaturation of particular essential amino acids as a byproduct with defective digestibility and poor nutritional value (Onifade et.al. 1998²⁹; Gupta et.al. 2006¹¹; Saha et.al. 2013³⁴). The other hydrothermal pretreatments adopted are thermo-chemical systems (including acidic hydrolysis and alkali hydrolysis), and steam pressure cooking. All of these consume more time, chemicals, and energy with high-priced industrial instruments for processing (Gupta et.al. 2006¹¹; Gurav et.al. 2013¹³; Saha et.al. 2013³⁴). Other techniques for feather disposing in practice include energy demanding incineration and landfilling, which further can contribute to environmental pollution (Khardenavis et.al. 2009¹⁷; Lateef et.al. 2010²¹; Brandelli et.al. 2010⁵). The poultry waste apart from polluting the environment is a cause for different human disorders including chlorosis, mycoplasmosis, and fowl cholera (Saha et.al. 2013). Currently applied feather incineration process has also been shown to increase the release of specific gases like CO, SO₂, Volatile Organic Compounds (VOCs), and Nitrogen Oxides into the environment. Hence, these pollutants result in respiratory diseases, cardiovascular diseases, and cancer, among other illnesses (Food &Water Watch Fact sheet, 2012). Therefore, there is a demand for managing feather waste by environmental friendly procedures.

Keratin is an important structural protein found extensively in the universe in the form of hair, feathers, nails, horns, hoofs, scales, and wool (Brandelli et.al. 2008⁶). The most important part of feathers is keratin, a mechanically stable chemically inert, and insoluble protein, which makes it hard to digest by most proteolytic enzymes. Feathers are composed of 90% by weight keratin and 10% is other components, 90% are resistive for degradation by common peptidases (Mazotto et.al. 2011²⁶). Keratin seen in epithelial tissues is classified as soft keratin, while keratin found in feathers, horns, hair, nails, and hoofs is hard (Navone et. al. 2018²⁸). The appearance of disulfide bonds in hard keratins is approximately more than that of soft keratins which is the basis for high rigidity and chemical resistance (Vidmar et.al. 2018⁴⁵). The larger percentage of sulfur, the higher is stability of keratin concerning solubilization (Maruthi Y Avasn et.al. 2011²⁵). High Sulfur content in keratin is because of sulfur-containing amino acids like cystine, cysteine, and methionine (Manirujjman et.al. 2016²⁴; Laba et.al. 2018¹⁹). One of the most environmentally beneficial way of managing CFW is by its breakdown employing keratinolytic bacteria to fertilizer and animal feed.

Keratinase is an extracellular enzyme produced at a wide range of pH and temperature which helps in breaking the disulfide bond of keratin during the process of degradation. Soil holds a number of phylogenetically distinct keratinophilic and keratinolytic microorganisms (Shah et.al. 2015³⁸). Keratinases are categorized into exoproteases and endoproteases based upon mechanism sites (Tork et.al. 2013⁴⁴). Enzymatic breakdown of feather waste is eco-friendly and also generates considerably important by-products (Papadopoulos et.al. 1986³³). The keratinases also find potential applications in industries including cosmetics, leather processing (tannery), fiber modification (textiles), detergents, modification of protein functionality (foods), biopolymers (films, coatings, glues), wastewater treatment/waste management, and bioconversion of keratinous wastes to methane or biohydrogen (Brandelli et. al. 2010⁵).

In the present work, screening, isolation, and identification of keratinase-producing bacterium from poultry soil is reported.

2. Materials and methods

2.1. Collection of soil sample

The soil sample was collected from the local poultry farms in sterile polythene bags and stored at 4°C, and processed for isolating keratin-degrading bacteria.

2.2. Collection and processing of chicken feather waste (CFW)

The white CFW was collected from poultry processing units in Karnataka, India. Then CFW was thoroughly washed under tap water to remove soil contaminants and continued washing with distilled water several times. Later all the materials were dried in a hot air oven. The dried feathers are chopped into small pieces (1mm) and powdered using a blender. This powder was stored in glass containers at room temperature, for further studies.

2.3. Isolation of CFW degrading bacteria

2.3.1. Screening for keratinase-producing bacteria

Soil sample (10g) is mixed with sterile saline (0.85%) and vortexed 10 min. Serial dilutions are made till 10⁻⁷. 1 ml of the diluted sample suspension from 10⁻⁷ dilutions was spread over feather enrichment media (FEM) plates (Cappuccino et.al. 1983⁷). The FEM plates were incubated at 45 (±2) °C for 3 - 4 days.

Alternatively, keratinase producer was grown in feather meal broth (FMB) g/L containing NaCl, 0.5; K₂HPO₄, 0.3; KH₂PO₄, 0.4 and feather (only carbon and nitrogen source), 10; pH 7.0. (El-Refai et.al. 2005⁸). The isolate from keratin agar plate was inoculated into FMB and placed on shaker incubator at 45 °C with a speed of 100rpm. The degradation of CFW was visualized physically and marked as a positive keratinase producer.

2.3.2. Rate of CFW Degradation

The keratinase-producing bacteria from FEM plate or FMB is inoculated into FMB medium having intact CFW pieces and the flasks are incubated at 45 °C in an incubator. After regular intervals, CFW degradation is determined by estimating the weight loss of CFW by following the equation below (Kani T et.al. 2012¹⁶).

$$\% \text{ of CFW weight loss} = [(\text{Initial CFW weight} - \text{Final CFW weight}) / \text{Initial CFW weight}] \times 100$$

2.3.3. Fourier transform infrared spectroscopy (FTIR)

The CFW degradation was assessed using FTIR analysis (Tesfaye et.al. 2017⁴³). The samples for FTIR (raw CFW and degraded CFW) were prepared by air-drying to remove moisture and cut into thin fine fibers.

2.4. Identification of CFW degrading bacteria

An isolated colony on FEM was picked up and submitted to Molecular Ecology Laboratory, NCCS-Pune for gene typing and phylogenetic analysis.

2.4.1. 16S rRNA Sequencing

The 16S rRNA gene is performed using primers 704F and 907R (Lane et.al. 1991²⁰) and sequenced in a 3730 DNA analyzer (Applied Biosystems-USA). The 16S rDNA sequences is generated using Chromas Pro and examined using online databases.

2.4.2. Phylogenetic analysis

The phylogenic tree is established in MEGA 11 software (Tamura et. al. 2021⁴²) and the evolutionary history was inferred by utilizing the Neighbor-Joining method (Saitou et.al. 1987³⁵). The evolutionary distances are computed in the Kimura 2-parameter method (Tajima et.al. 1984⁴⁰).

Assay for keratinolytic activity

FMB medium (90 ml) is taken in 250 ml Erlenmeyer flask, 10 ml of overnight grown culture was used as inoculum and incubated at 45°C in rotary shaker (100 rpm) for 5 days. Control, FMB medium without any inoculum. Enzyme free from un-degraded feather was obtained by filtering the culture medium through Whatmann No. 1. The filtrate is subjected to centrifugation at 10000 rpm for 10 min at 4°C to remove any bacterial matter. The supernatant obtained was assessed for keratinase activity. Keratinase activity was assayed (Sangali et.al 2000³⁷) with azocasein as the substrate. The reaction mixture contains 1ml of enzyme and 1ml of azocasein (4mg/ml) in 50Mm Tris buffer pH 8.0. This mixture is incubated at 55°C in water bath for 15 min. The reaction was stopped by adding 2 ml of 10% TCA. The absorbance of supernatant at 440nm is monitored as keratinase activity. One Unit of enzyme activity refers to a change of absorbance of 0.01 at 440nm in 15 min at 55°C.

$$\text{Enzyme Units per ml} = \text{OD} \times 4 \times \text{dilution factor} / 0.01 \times T$$

Where, T=incubation time, 4=total volume

Protein Estimation

Protein concentration from the CFS was determined by the method (Lowry et.al. 1956²³).

Estimation of amino acids

Amino acid estimation was carried out by the Ninhydrin assay as per the reported protocol (Moore et.al. 1948²⁷).

3. Results

3.1. Isolation of keratinase-producing bacteria

The soil suspension was prepared and spread over FEM and incubated. After 3 days of incubation keratinase-producing colonies were observed (Fig.1).

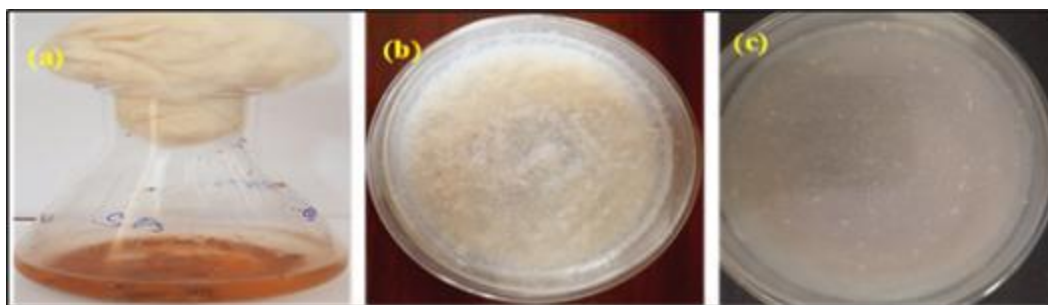


Figure 1 (a) Saline suspension of poultry soil, (b) FEM showing growth of bacteria, at 45 °C, (c) Control plate, at 45 °C

3.2. Assessment of keratinase producers

The bacterial colonies from FEM plate were assessed for efficiency of CFW degradation in FMB medium. The CFW was completely removed in 5 days. Further, the feather barbules and rachis were degraded slowly (Fig.2). The efficiency of CFW degradation was found to be 19%.

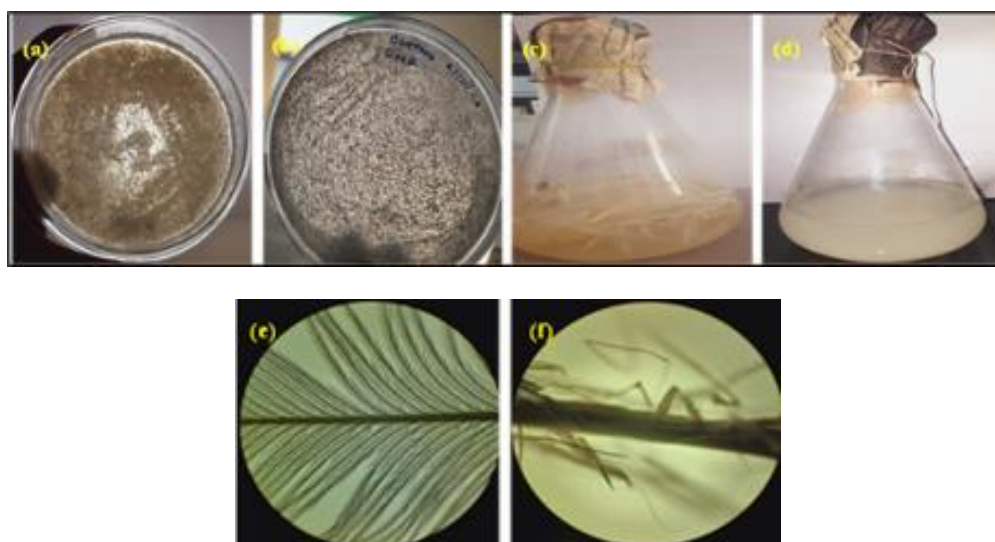


Figure 2 (a) FEM plate showing growth of CFW degrading bacteria at 45 °C, (b) Control FEM plate showing no growth at 45 °C, (c) Control FMB medium at 45 °C, (d) FMB with keratinase producer at 45 °C, (e) Undegraded CFW under microscope (4x) after 5 days, (f) Degraded CFW under microscope (4x) after 5days

3.3. FTIR analysis of CFW

CFW degradation by the bacterium is achieved by removal of functional groups in feather proteins. The FTIR spectrum of the undegraded and degraded CFW is given in Fig. 3. The samples are scanned from 400 to 4000 cm^{-1} to give characteristic peaks of peptide bonds (CO-NH). The main functional groups present in the keratin are amine and carboxylic groups. The peaks at 3276 ($-\text{NH}_2$), 2930 ($-\text{CH}$), 1631 ($-\text{OH}$), 1626 ($-\text{NH}_2-\text{C}=\text{O}$), 1528 (bending mode of amide), and 1520 ($-\text{NO}$) cm^{-1} were observed in the undegraded CFW (fig.3). while, in the case of degraded CFW, the percentage of Transmittance of all the characteristic peaks was considerably decreased, and even some peaks disappeared. In summary, the declined transmittance at 1631 cm^{-1} reports the α -helical structure equivalent to Amide I (Sharma et.al. 2022³⁹). The peaks between 1570 and 1720 cm^{-1} are characteristics of the β -sheet conformation of feather keratin (Poole et.al. 2011³²). These results, also support the degradation of CFW.

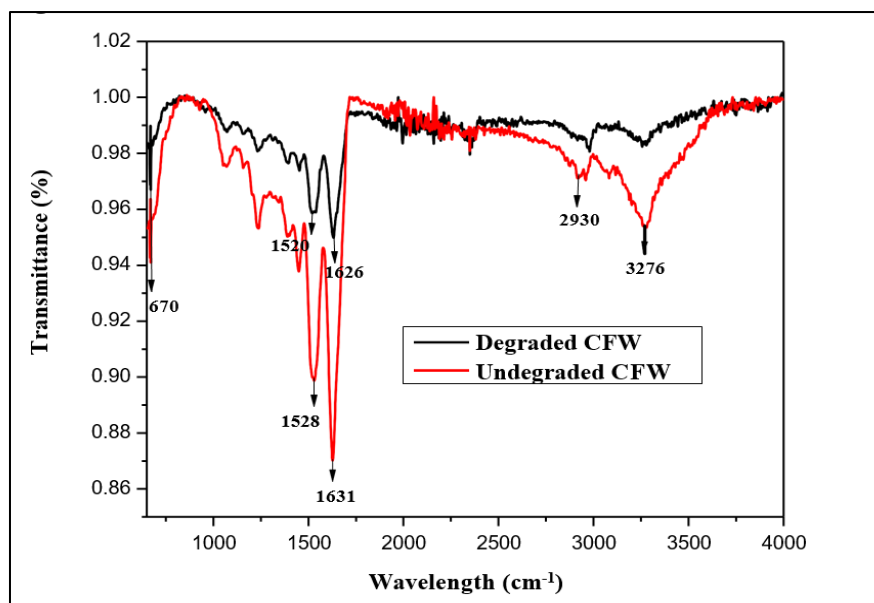


Figure 3 FTIR profile of CFW degradation with peaks showing bond breakage

3.4. Molecular identification of CFW-degrading bacteria

3.4.1. 16s rRNA sequencing

The analysis is derived in a single 16S rRNA gene sequence of 1363 bp of the isolate with its closest type strains in the database. CFW degrading bacterial isolate is deposited in GeneBank data base (fig.4) under the accession number PP059639.

3.4.2. *Paenibacillus koreensis* strain YC300 16S ribosomal RNA, partial sequence

AATCCTTCTACGGACAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCTTGGGGAGT
TCCTTCGGGAATCCCCGGGAGCGGCGGACGGGTGAGTAACACGTAGGCAACCTGCCTGTAAGACCGGGATAACTACCGGAAACG
GTAGCTAAGACCGGATACGTGGCCTTCTCGCATGACAGGATCAACAAACACGGGGTAACCTGTGGCTTACAGATGGGCCTGCGG
CGCATTAGCTAGTTGGTGGGTAACGGCTCACCAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTG
AGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAG
TGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGTCGCGGAGAGTAAGTCTGCTGCGAATGACGGTACCTGA
GAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGC
GCGCGCAGGCGGCGCTTAAGTTCTGGTGTTTAAGCCCCGGGGCTCAACCCCGTTGCGACTGGAAACTGGGCGGCTTGAGTGCAG
GATAGGAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAAGCGGCTTTCTGGCC
TGTAAGTACGCTGAGGCGCGAAAGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTACACGATGAGTGCTA
GGTGTAGGGGTTTCGATACCCTTGGTGCCGAAGTAAACACAATAAGCACTCCGCCTGGGAGTACGCTCGCAAGAGTGAAACT
CAAAGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATGTGGTTTAATTGCAAGCAACGCGAAGAACCTTACCAGGTCTTGAC
ATCCCTCTGAATATCCTAGAGATAGGGTAGGCTTTCCGGACAGAGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTGAACCTTAGTTGCCAGCATTAAGTTGGGCACTCTAAGTTGACTGCCGG
TGACAAACCGGAGAATGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTAGTACAATGGCCGGTAC
AACGGGAAGCGAAGTCGCGAGATGGAGCCAATCCTAAGAAAGCCGGTCTCAGTTCCGATTGCAGGCTGCAACTCGCCTGCATGA
AGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGA
GAGTTTACAACACCCGAAGTCGGTGGGGTAACCGCAAGGAGCCAGCCGCCGAAGGTGGGGTAGATGATTGGGGTGAAGTGGA
ACAAGGTAGCCGTACCGGAAGGTGG (1536bp)

The closest phylogenetic neighbor found for the sequence is *Paenibacillus koreensis* YC300. (Fig.5)

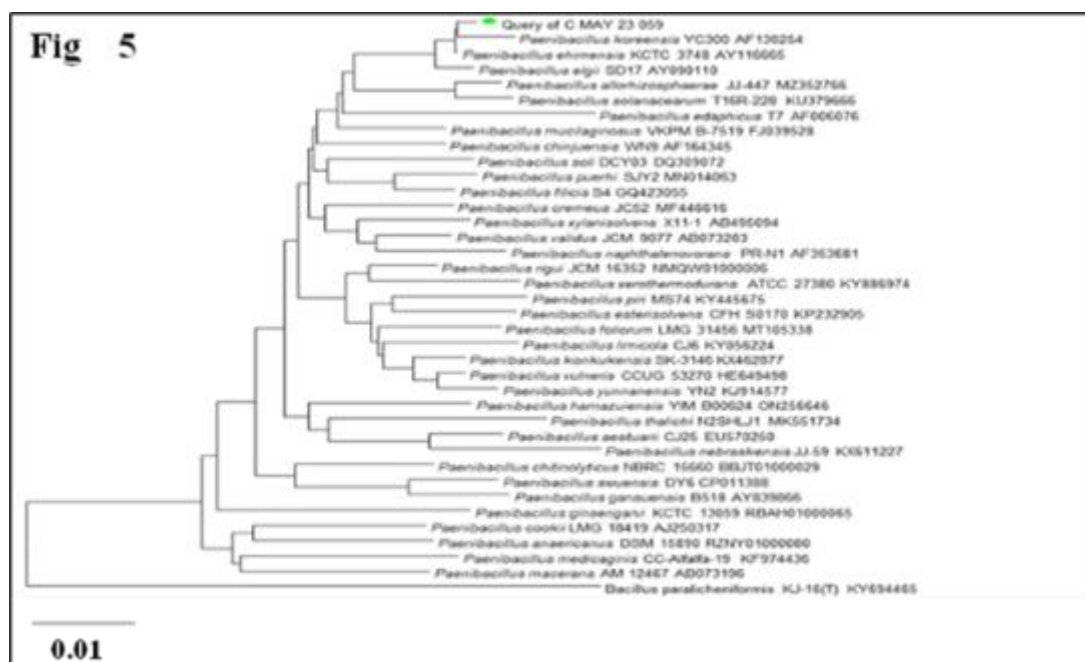


Figure 5 Phylogenetic tree of keratinase bacterium

3.5.1. Keratinase assay

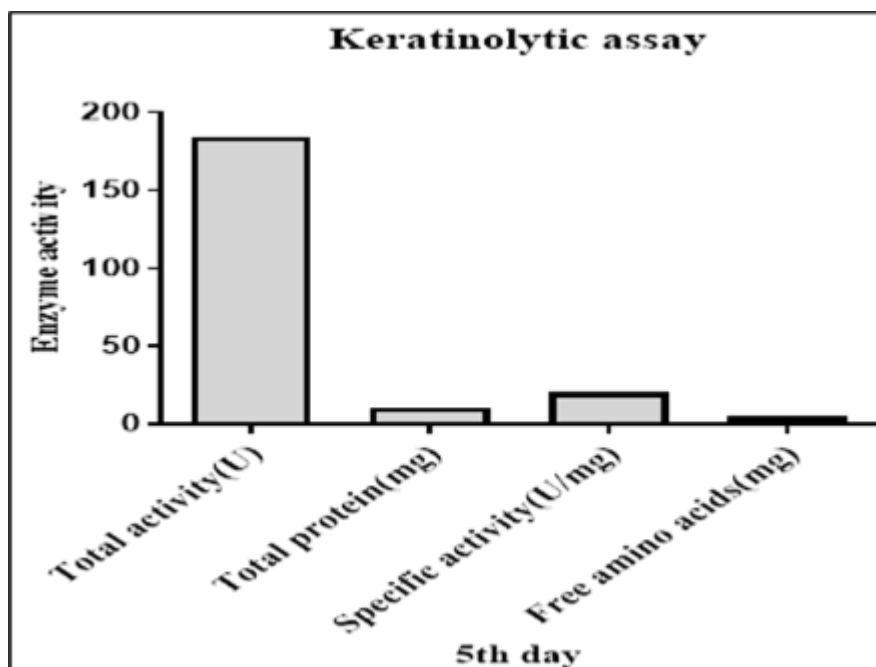


Figure 6 Keratinase activity

4. Discussion

Accumulation of CFW is increasing every year, keratin protein present in CFW is unavailable for natural degradation. There are some familiar procedures to treat CFW which include burning, land dumping, high pressure heating, and chemical breakdown, which require large amounts of force, these diminish some important amino acids present in chicken feathers and are non-eco-friendly. Because of all these issues, evolving eco-friendly methods for the degrading CFW has turned into vital importance. CFW degradation using microorganism owns a greater advantage and it is significantly investigated in modern times. Different species of bacteria, fungi, and actinomycetes are identified as good keratin degraders.

Our experiments were successful in isolation and identification of bacterium involved in CFW degradation. In earlier reports many *Bacillus* species have exhibited the capacity to degrade CFW, which include *B. licheniformis* (Xu et.al. 2009⁴⁶), *B. pumilus* (Fakhfakh-Zouari et.al. 2010⁹), *B. altitudinis* (Kumar et.al. 2011¹⁸), *B. megaterium* (Anitha et.al. 2012²), *B. cereus* (Jeevana Lakshmi et.al. 2013¹⁵), *B. subtilis* (Gupta et.al. 2014¹²) and *B. safensis* (Lateef et.al. 2015²²). These observation is in line with the fact that the nature in general mobilizes unusual substrates in their environment to their nutritive requirements.

The CFW degrading bacteria are profoundly found in keratin-rich environments (Habbechhe et.al. 2014¹⁴). Soil sample taken from poultry land provided bacterium with the capacity to degrade CFW on FEM plates at 45°C. The rate of CFW degradation was followed in FMB in liquid cultures, since the organism shows degradation at 45°C, considered to be highly stable at high temperatures. The experiments showed that CFW was rapidly degraded to powder form within 5 days, there after only feather barbules and rachi were degraded slowly. Further, to understand the changes during CFW degradation and structural alterations FTIR analysis was performed, where the changes in the location of peaks leads to conformational modifications in keratin. Here bacterium degrading CFW was identified as *Paenibacillus koreensis* YC300 by 16S rRNA gene sequencing and phylogenetic studies. *Paenibacillus* genus is equivalent to *Bacillus* (Ash et.al. 1993³). The literature survey indicates only few reports related to CFW degradation by *Paenibacillus*, and one among is *Paenibacillus woosongensis* TKB2, shown 87 % degradation CFW (0.8 %, w/v), after optimizing culture conditions, in 2 days (Paul et.al. 2014³¹), but *Paenibacillus koreensis* YC 300 showed keratinase activity of 18.75 U/mg and free amino acids of 3.209 mg and % of feather degradation is found to be 19%.

The advantage of keratinolytic microorganisms and their enzymes are twofold. Primarily they can solubilize and eliminate hardly degradable substrates like keratin and secondly they can convert ample undegraded CFW into a

nutritionally rich meal (peptides and amino acids), that shows applications in organic fertilizer and animal feed supplement (Bhari et.al. 2018⁴).

5. Conclusion

To date, numerous studies have been undertaken on the isolation and identification of bacteria with keratin degradation properties. The study reports successful isolation and identification of a novel strain *Paenibacillus koreensis* YC300 which degrades CFW. The *P. koreensis* YC300 induces an extracellular keratinase for the degradation of keratin rich CFW. The literature survey points out that *Paenibacillus* members can improve crop growth by, biological nitrogen fixation, phosphate solubilization, production of phytochrome IAA, and release of siderophores which enable iron acquisition. Hence, *P. koreensis* YC300 can be harnessed for the biotechnological control of CFW through efficient biodegradation.

Compliance with ethical standards

Acknowledgments

The authors thank the Department of Biochemistry, Bangalore University, Bangalore for providing the facilities to carry out this research. Finally, I would like to thank Nandisha P S, Department of Chemistry, Bangalore University for their valuable input.

Disclosure of conflict of interest

Authors declare no conflicts of interest.

Author contributions

Sneha M J: Experimental work, Work design, original draft writing. C. S. Karigar: Work designing, Editing, and revision of the draft. Suneetha P: Editing, revision of the draft, and Formal analysis.

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