



Eco-friendly Feather Waste Degradation *via* Termite-Associated Bacteria

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ABSTRACT

Chicken feathers, a major waste product of the poultry industry, create significant environmental challenges due to their large volume. Converting this waste into useful products is crucial for sustainability. This study highlights the keratinolytic potential of *Bacillus subtilis* (Bs), *Bacillus cereus* (Bc), and *Bacillus thuringiensis* (Bt), all isolated from termite guts, for the effective degradation of chicken feathers. These bacteria exhibit strong keratinolytic activity, enabling them to break down the resilient keratin protein found in feathers. Each species demonstrated its ability to degrade feathers, with Bs achieving this in eight days, Bc in eleven days, and Bt in thirteen day. However, optimizing conditions, particularly maintaining aerobic environments and pH at 7 ± 0.5 , enabled faster degradation using a bacterial consortium. This consortium achieved complete feather degradation in just five days. The resulting feather lysate has potential applications in biofertilizers, animal feed, and pharmaceuticals. This research underscores the efficiency and sustainability of biological methods, particularly bacterial consortia, in feather waste bioconversion, offering an eco-friendly solution for large-scale waste management.

Keywords: Chicken feather; Keratin; Termites; Keratinolytic bacteria; Degradation

INTRODUCTION

Chicken feathers, a significant waste product of the poultry industry, pose a serious environmental challenge due to their large volume. Each bird typically produces up to 125 grams of feathers, contributing to an estimated 3,000 tons of feather waste generated globally each week (Prasanthi et al., 2016). The disposal of large quantities of feather waste presents a significant global environmental challenge. Untreated feather waste not only poses risks to public health and the environment but also provides a habitat for pathogenic microbes, which can proliferate and contribute to environmental contamination. Additionally, the natural degradation of feathers releases harmful pollutants, including nitrous

oxide, ammonia, and hydrogen sulfide, further exacerbating environmental concerns (El-Gindy et al., 2023). Chicken feathers consist of approximately 85% crude protein, including 70% amino acids, along with vitamins, essential elements, and growth factors. However, due to their mechanical stability, feathers are resistant to hydrolysis by proteolytic enzymes. This resistance is primarily due to the high keratin content, a rigid protein that imparts structural stability. The keratin's structure is further reinforced by disulfide bonds between peptide chains, creating polymeric networks with hydrophobic interactions and hydrogen bonding, resulting in significant mechanical strength. Therefore, efficient degradation is crucial to minimizing their environmental impact and creating valuable by-products. (Kamran, 2022)

Three primary approaches for feather degradation-physical, chemical, and biological-are discussed in the previously reported in the literature. Physical methods such as puffing and pressurized hydrolysis, and chemical methods involving acids or alkalis, have been explored. However, these techniques are energy-intensive and can lead to significant product degradation, limiting their applicability. In contrast, biological methods, particularly those involving keratinolytic bacteria, have emerged as more eco-friendly and economically viable solutions for converting feather waste into useful by-products without compromising product quality (Peng et al., 2019; Yadav et al., 2021).

In the biological degradation process, keratinolytic bacteria offer a highly efficient, eco-friendly, and economical method to convert feather waste into lysate, which is nutritionally valuable. Feather keratin-degrading abilities in bacteria had been observed mostly in the *Bacillus* sp. (Cai et al., 2008). *Bacillus* species, particularly those isolated from termite guts, have demonstrated significant keratin-degrading abilities due to the presence of keratinase enzymes. Termite guts harbor a rich diversity of both aerobic and microaerophilic bacteria (König, 2006), many of which possess the enzymatic capability to break down the robust keratin proteins in feathers, making them ideal for sustainable bioconversion processes.

In this study, efforts were made to isolate and identify *Bacillus* species from the termite gut, which were then employed for the complete degradation of chicken feathers. HiCrome Bacillus Agar was utilized to selectively isolate *Bacillus* species. The isolated

bacteria were identified through various techniques, including cultural and morphological analysis, along with 16S rRNA gene sequencing. These identified *Bacillus* strains were then used for feather degradation experiments, demonstrating their potential in bioconversion applications.

MATERIAL AND METHODS

Field Sampling and Collection Methods for Termites

Termites for isolating bacterial species were collected from Ambazari Biodiversity Park in Nagpur, Maharashtra. This location was selected due to its tropical climate and high levels of biodiversity, which are conducive to the presence of diverse microbial species. (Pervez, 2018). Termite nests were located on dry tree bark, and careful collection was performed using a sterile wooden stick to minimize contamination. Then, they were transferred to a laboratory in a packed box. Medium- to small-sized termites that were collected from the park exhibited a reddish-brown colouration and a distinct separation between the head and body segments.

Isolation of Bacteria from Termite's Gut

The termites were placed in sterilized Petri plates, washed with sterile distilled water, and then sterilized with a 70% alcohol solution for 30 seconds. The body parts of the sterilized termites were separated from their heads, crushed in a test tube with a sterile glass rod, and mixed with 1 ml of sterile distilled water and subjected to serial dilution for bacterial extraction. Isolation of bacteria from the termite's gut was performed by crushing the body parts of termite's. HiCrome Bacillus Agar, which is specific for *Bacillus* species isolation was used (Alippi, et al., 2019). 100 µl, 80 µl, 60 µl, 40 µl and 20 µl of suspension of crushed termite gut were then spread on already prepared HiCrome Bacillus Agar plates with the help of a sterilized spreader and the plates were kept in incubator at 37°C for 24 hours for growth. The specific colonies of particular organisms on the HiCrome Bacillus agar plates were selected and further cultured on this specific media in order to obtain the pure culture of bacteria.

Identification of Bacillus species

The isolates were initially spread on Hi-Crome Bacillus Agar Plate and the colonies were tried to be identified and characterised on the basis of its morphological and cultural patterns and three selected colonies were

labelled as OB1, OB2 & OB3 as mentioned in table 1. The resulting colonies were subjected to quadrant streaking for obtaining pure cultures and further proceeded for Gram staining. Finally, molecular identification of the *Bacillus species* was performed using 16S rRNA sequencing.

Morphological Identification:

Bacterial isolates were analysed for Gram character and morphology by preparing heat-fixed smears from isolated colonies on glass slides. Following the standard four-step staining protocol utilizing crystal violet, iodine, decolorizer, and safranin—the slides were rinsed and air-dried. Finally, the samples were examined under a microscope to differentiate the bacteria based on their staining reaction and cellular shape (Table 2).

Cultural Identification

To identify the isolates, colonies were cultured on HiCrome Bacillus Agar, a specialized chromogenic medium. This selective agar allows for the rapid differentiation of *Bacillus* species based on distinct colony colour changes triggered by specific enzyme reactions (Table 3).

Molecular Identification

To identify the bacterial isolates, genomic DNA was extracted from the overnight cultures using CTAB/NaCl protocol (Wilson, 2001). The 16S rRNA gene was subsequently amplified through Polymerase Chain Reaction (PCR) using universal primers (Forward: 5'-AGAGTTTGATCCTGGCTCAG-3'; Reverse: 5'-GGTTACCTTGTACGACTT-3'). The amplification process was initiated with a denaturation for 5 min at 94°C, followed by annealing for 1 min at 55°C, and extension for 2 min at 72°C, repeated for 30 cycles. Additionally, there was a final extension for 5 min at 72°C. The sequencing was carried out in ABI prism 3100 Genetic Analyzer (Applied Biosystems). The sequences were checked against the microbial nucleotide databases using BLASTN search algorithms and phylogenetic analysis was performed by COBALT Multiple Sequence Alignment.

Feather Degradation

Feather degradation is a critical process for waste management and environmental sustainability, as it facilitates the conversion of keratin-rich materials into reusable forms through microbial activity. To enhance the bacterial population for this purpose, an inoculum

build-up procedure was executed. A single bacterial colony was aseptically transferred from a plate into 10 ml of nutrient broth and incubated overnight for initial growth. Subsequently, 10 ml of this bacterial culture was added to 90 ml of sterile nutrient broth to expand the inoculum volume and cell density further. White broiler chicken feathers were collected from a local market, meticulously washed with tap water to remove macroscopic debris, and rinsed with distilled water to eliminate any remaining impurities. The feathers were then dried in an oven to remove residual moisture. The dried feathers were cut into smaller pieces, approximately 2-3 cm long, to increase the surface area for microbial degradation. A 400 ml volume of feather degradation medium was prepared by dissolving 0.4 g of NH₄Cl, 0.4 g of NaCl, 0.24 g of K₂HPO₄, 0.32 g of KH₂PO₄, and 0.08 g of yeast extract in 400 ml of sterile distilled water (Godbole, et al., 2017) (Kim et al., 2001) (Yadav et al., 2021). Four grams of the prepared feather pieces were added to the medium, and the pH was adjusted and maintained between 7.0 and 7.5 to optimize bacterial activity, as an appropriate pH is crucial to the preservation of feathers (Barman et al., 2017). The degradation process was conducted for 5 to 13 days depending on a particular strain of the bacteria, maintaining aerobic conditions with constant stirring in four different experimental sets as tabulated in table 4.

RESULTS AND DISCUSSION

Identification of the bacterial isolates

The Hi-Crome Bacillus agar plates showcased distinct colony morphology after incubation. Isolates were grown well and produced different colonies with different colours and morphology. Characteristics of *Bacillus species* obtained are shown in table 4. Pure culture of selected bacteria was obtained by four-way streaking of selected bacteria displayed different coloured colony characters (Figure 1). Further performing 16S rRNA study (details ahead) and on the basis of morphological and cultural characteristics it was confirmed that isolate OB1 was *Bacillus thuringiensis*, OB2 was *Bacillus cereus* and OB3 was *Bacillus subtilis*.

Molecular Identification and Phylogenetic Analysis

To determine the taxonomic identity of the bacterial strain, molecular identification was performed using 16S rRNA gene sequencing. Genomic DNA was extracted from the isolate following standard

protocols, and the 16S rRNA gene was amplified via PCR using universal bacterial primers. The resulting amplicon was then purified and sequenced (Figure 2, 3 & 4). The obtained 16S rRNA gene sequence was analyzed using BLAST against sequences available in public databases, such as NCBI GenBank, to identify closely related bacterial taxa. For phylogenetic analysis, multiple sequence alignment was conducted using software like ClustalW, and a phylogenetic tree

was constructed using MEGA 7 software with either the neighbor-joining or maximum likelihood method. The phylogenetic analysis revealed that the OB1, OB2 and OB3 strain clusters closely with members of the *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus subtilis* respectively (Figure 5). This taxonomic placement is consistent with previously reported studies, confirming the evolutionary affiliation of the isolate.

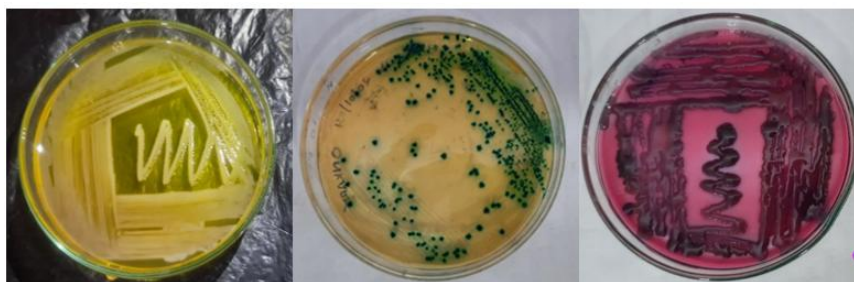


Figure 1. Bacterial Isolates OB3, OB2 and OB1 respectively (Table 3).

Table 1. Characteristics of *Bacillus* Species

Codes	Observation	Colony morphology
OB3	Yellow coloured colonies	Mucoid colonies, spread colonies
OB2	Light blue to green tiny colonies	Rounded colonies with regular margins
OB1	Blue coloured colonies	Flat colonies with irregular margins

Table 2. Morphological characteristics of *Bacillus* species

Characteristics	OB3	OB2	OB1
Gram Reaction	Gram +ve	Gram +ve	Gram +ve
Shape	Rod	Rod	Rod
Motility	Motile	Motile	Motile

Table 3. Colony characteristics of *Bacillus* species on HiCrome Bacillus Agar

Sr No	Isolates	Characteristics
1	OB3	Yellowish Colonies
2	OB2	Light blue to green tiny colonies
3	OB1	Light blue, large, flat mucous colonies with irregular margins.

Table 4. Experimental sets for the feather degradation studies.

Sr No	Flask	Sets of Experiment
1	A	4g feathers, 400ml Feather Degrading Media & 40ml OB3 strain
2	B	4g feathers, 400ml Feather Degrading Media & 40ml OB2 strain
3	C	4g feathers, 400ml Feather Degrading Media & 40ml OB1 strain
4	D	4g feathers, 400ml Feather Degrading Media & 13ml of each OB3 strain OB2 strain OB1 strain

AATGGAGAGTTTGA TCCTGGCTCAGGATGGCGGCGTGCCATAACATGCAAGTCGAGCGAATG
 GATTAAGAGCTTGCTCTTA TGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAA
 CCTGCCCAATAAGACTGGGATAACTCCGGGAAACCGGGCTAATACCGGATAACATTTTGAACC
 GCATGGTTTCGAAA TTGAAGGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCG
 CATTAGCTAGTTGGTGAGGTAAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGG
 TGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGG
 GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCGCGTGAGTGATGAAGGC TTTCG
 GGTCGTAAAACTCTGTTGTTAGGGAAGAACCAGTGC TAGTTGAATAAGCTGGCACCTT
 GACGGTACCTAACCCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAA TACGTAGGT
 GGCAAGCGCTATCCGGAA TTA TTGGGCGTAAAGCGCGCTGGTTTCTTAAGCTGTATGT
 GAAAGCCCAACGGCTCAACCGTGGAGGGTCA TTGGAAACTGGGAGACTTGAGTGCAGAAAGAGG
 AAAGTGGAA TTCCATGTGTAGCGGTGCGTAGAGATATGGAGGAACACCAAGTGGCGAAG
 GCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTA GA
 TACCCCTGGTAGTCCACGCGGTAAACGATGAGTGC TAAGTGTTAGAGGGTTTCCGCCCT
 TTAGTGCTGAAGTTAACGCA TTAAGCACTCCGCTGGGGAGTACGGCCGCAAGGCTGAAACTC
 AAAGGAA TTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAA TTCGAAGCAA
 CGCGAAGAACCTTACCAGGTCTTTGACATCCCTGACAACCC TAGAGATAGGGCTTCTCCTTCG
 GGAGCAGAGTGACAGGTGGTGCA TGGTTGTCGTCAGCTCGTGCTGTAGATGTTGGG
 TTAAGTCCCGCAACGAGCGCAACCC TTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGG
 TGACTGCCGGTGACAAAACCGGAGGAAGGTGGGGATGACGTCAAA TCATCATGCCCC
 TTATGACCTGGGCTACACACGTGCTACAA TGGACGGTACAAAGAGCTGCAAGACCGCGTAATC
 TCATAAAACCGTTCTAGTTCCGATTG TAGGCTGCAACTCGCCTACATGAAGCTGGA
 ATCGCTAGTAA TCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCCTTG TACACACCGC
 CCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTGGAGC
 CAGCCGCTTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAAACAAGGTAGCCGTATCGGAAG
 GTGCGGCTGGATCACCTCCTTTCTATGGAGAA TTGATGAACGCTGTTCATCAATATAA
 GTTTCGCTGTTTCGTTTGTTCAGTTTGAACG

Figure 2. The obtained 16s rRNA sequence of *Bacillus thuringiensis* (OB1 isolate)

TGCAGGTGGTTTCTTAAAGTCTGATGTGAAAGGCCACGGCTCAACCGTGGAGGGTCA TTGGA
 AACTGGGAGACTTGAGTG CAGAAGAGGAAAGTGGAA TTCCATGTGTAGCGGTGAAAT
 GCGTAGAGATATGGAGGAACACCAAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGA
 GGGCGGAAAGCGTGGGGAGCAAACAGGATTAGATACCC TGGTAGTCCACGCGGTAAAC
 GATGAGTGCTAAGTGT TAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCCCTCGTGTCTGTA
 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC TTGATCTTAGTTGCCATCATTT
 AGTTGGGCACTCTAAGGTATTAAGACTCCGCTTGGGGAGTACGGCCGCAAGGCTGAAACTCA
 AAGGAA TTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG
 CGAAGAACC TTACCAGGTCTTGACATCCCTGACAACCC TAGAGATAGGGCTTCTCCTTCGGG
 AGCAGAGTGACAGGTGGTGCA TGGTTGTCGTCAGGACTGCCGATATACATGCCCTT
 ATGACCTGGGCTACACACGTGCTACAA TGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGG
 AGCTAATCTCATAAAACCGTTCTAGTTCCGATTG TAGGCTGCAACTCGCCTACATGA
 AGCTGGAA TCGCTAGTAA TCGCGGATCAGCATGTACGCGGGTGAATACGTTCCCGGGGCCCTT
 GTACACACCGCCCGGTACACCCACGAAGAGTTTGTAACACCCGAAAGTCGGTGGGG
 TAACCTTTTTGGAACCCAGCCCGCTTAAGGTGGGAACAAATGATTGGGGTGAGTTACACAAGG
 GGGGGGGGCCACACACAAGGGGGGTGTTCTAAGCC

Figure 3. The obtained 16s rRNA sequence of *Bacillus cereus* (OB2 isolate)

GCGCACTGGCTCAGGACGGCGTGCTTAATACATGCAAGTCGAGCGGACAGATGGGAAGCTTG
CTCCCTGATGTAAAGCGGCGGACGGGTGAGTAACACGTGGGTAACTGCCTGTAAGACT

GGGATAACTCCGGGAAACCGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACAT
AAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGTTAGCTAGTTGGTGAGGT

AACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACT
GAGACACGGCCAGACTTCTACGGGAGGCAGCTAGGGAATCTTATCCGCAATGGAC

GAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTGGATCGTAAAGCTCTGTTGT
TAGGGAAGAACAAAGTACCCTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAA

AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAA
TTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGC

TCAACCGGGGAGGGTCAATTGGAACCTAAAGGGGAACCTTGAGTGCAGAAGAGGAGAGTGGAA
TCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCACTGGCGAAGGCGAC

TCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCTTAGT

GCTGCAGCTAACGCACTTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAG
GAAATTGACGGAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACCGCAAGAACCCTTAC

GCCAGGCTTTGACATCCTCTGACAACTCCTAGAGATAGGACGTCCCTTCGGGGGCAGAGTGAC
AGGTGGTGATGGTTGTCGTACGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAA

CGAGCGCAACCCCTTGATCTTAGTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCAAATCATATGCCCTTATGACCTGGGCTACACAACAA

TGGACAGAAACAAAGGGCAGCGAAACCGCGAGGTTAAGCGTTCTCAGTTCGGATCGCAGTCTG
CAACTCGACTGCGTGAAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGA

ATACGTTCCCGGGCCCTTGATACACACCGCCCGTCACACCAGAGAGTTTGTAACACCCGAAGTC
GGTGAGGTAACTTTTAGGAGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAA

GTCGTAAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATC

Figure 4. The obtained 16s rRNA sequence of *Bacillus subtilis* (OB3 isolate)

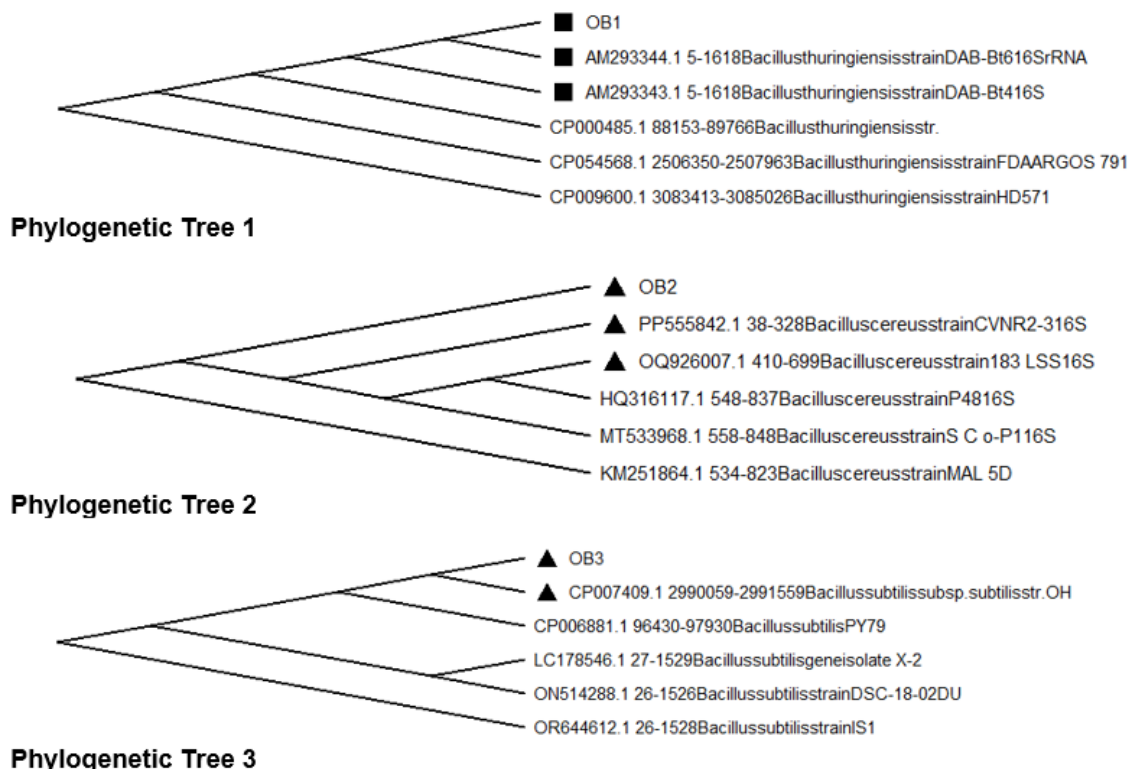


Figure 5. The phylogenetic tree 1, 2 & 3 reveal that the OB1, OB2 and OB3 strain clusters closely with members of the *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus subtilis* respectively

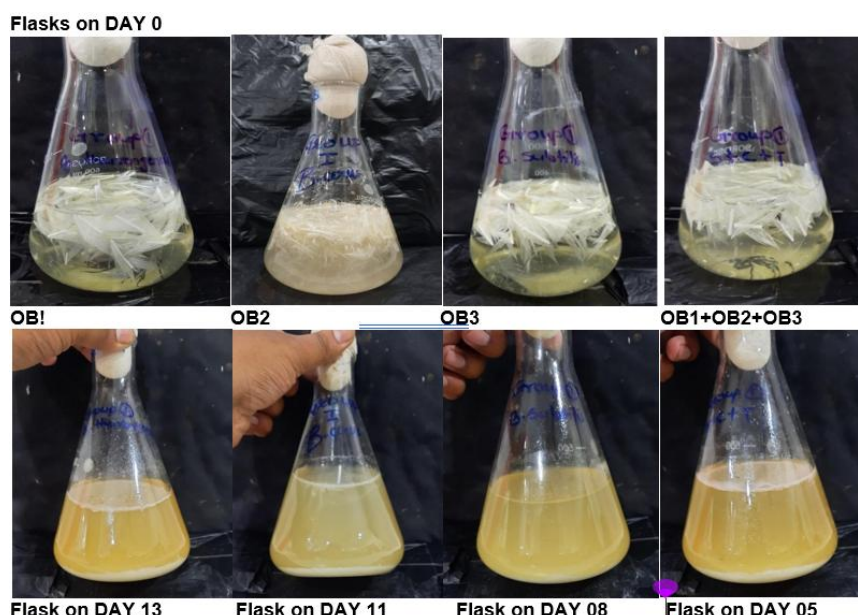


Figure 6. The feather degradation by three different bacterial isolates from termite gut that have been identified on the basis of morphology, cultural and 16s RNA studies as coded as OB1, OB2, OB3 were observed for 8-13 days with negative control for 20 days.

Feather Degradation

Negative results were obtained from the 1st set of experiments as no feathers were degraded after 18-20 days. Feathers condition was same as that on Day 1 of incubation. The obtained result may be because of a lack of oxygen in the flask as the flasks were airtight with a cotton plug and may be due to a shift in pH due to the activity of bacteria present. pH when checked was ranging between 4.3-4.5. Considering the previous result some changes were made later and positive results were shown by all four flasks as the chicken feathers were degraded completely. A flask with *Bacillus subtilis* degrades the feather completely in 8 days, a flask with *Bacillus cereus* degrades the feather in 11 days while a flask with *Bacillus thuringiensis* degrades the feather completely in 13 days after incubation and the flask with consortium of three bacterial culture showed complete degradation of chicken feather in 5 days (Figure 6). The major changes that were made in 2nd set of experiment was that the flasks were kept open daily for 5-10 minutes to supply oxygen for the bacteria present and pH was kept checking after every 24 hours. pH was maintained at the range of 7-7.5 throughout the experiment.

The present study successfully identified the bacterial isolate as *Bacillus thuringiensis* (OB1) through 16S rRNA gene sequencing, a widely accepted molecular approach for bacterial taxonomy and phylogenetic analysis. The obtained sequence showed high

similarity to reference sequences of *B. thuringiensis* available in NCBI databases, confirming the identity of the isolate. The use of 16S rRNA gene sequencing as a reliable tool for identifying *Bacillus* species has been previously validated in numerous studies. For instance, Liu et al. (2017) demonstrated the effectiveness of 16S rRNA gene analysis in distinguishing *B. thuringiensis* from closely related species such as *B. cereus* and *B. anthracis*, emphasizing the role of hypervariable regions in precise taxonomic resolution. Similarly, Swiecicka et al., (2006) reported that partial sequencing of the 16S rRNA gene was sufficient to differentiate environmental isolates of *B. thuringiensis* with high confidence. Our findings are also in agreement with the study conducted by Nakamura (1994), who performed a comprehensive phylogenetic analysis of the *Bacillus cereus* group and affirmed that *B. thuringiensis* could be accurately distinguished based on molecular signatures present in the 16S rRNA sequence. Although certain genetic markers such as cry genes are commonly used for *B. thuringiensis* characterization, the 16S rRNA gene provides a broader and foundational framework for its classification, especially when corroborated with phylogenetic analysis and database comparison.

The isolate OB2 under investigation was identified as *Bacillus cereus* through 16S rRNA gene sequencing. The obtained sequence shared high similarity with *B. cereus* reference strains in the NCBI GenBank

database, supporting its taxonomic assignment. The *Bacillus cereus* group is phylogenetically close to *Bacillus anthracis* and *Bacillus thuringiensis*, making precise molecular identification important. Liu et al. (2017) emphasized that although the 16S rRNA gene alone cannot differentiate some members of the *B. cereus* group at the species level due to high sequence similarity (>99%), alignment with database sequences and phylogenetic analysis still provide reliable classification for typical environmental and clinical isolates. In a study by Jackson et al. (1995), partial 16S rRNA sequencing was employed to delineate species within the *B. cereus* group. They reported that while the gene alone may not distinguish between *B. cereus* and *B. thuringiensis*, the presence of specific nucleotide differences combined with phenotypic and ecological data enhances identification reliability. Our isolate showed sequence alignment and divergence patterns consistent with *B. cereus*, confirming its identity. Helgason et al. (2000) analyzed multiple isolates and proposed that molecular markers, including 16S rRNA, in combination with toxin profiling, can clearly resolve *B. cereus* from other closely related species. The presence of distinctive rRNA motifs and alignment with known *B. cereus* strains in our analysis corroborates this approach.

The 16S rRNA gene sequence obtained in this study enabled the molecular identification of the isolate OB3 as *Bacillus subtilis*. The sequence analysis revealed high similarity with reference strains of *B. subtilis* in public databases, confirming its taxonomic classification. Several studies have supported the efficacy of 16S rRNA sequencing in identifying *B. subtilis* from environmental and industrial samples. Roohi et al. (2012) demonstrated the successful use of partial 16S rRNA sequencing to identify *B. subtilis* isolates from Himalayan soil samples. Their findings emphasize the utility of this method for strain-level identification, especially when supplemented with biochemical or morphological data. Similarly, in a comparative study, Mandic-Mulec et al. (2015) highlighted the phylogenetic distinctiveness of *B. subtilis* within the *B. subtilis* species complex, based on 16S rRNA gene-based and whole-genome analyses. Singh et al. (2020) confirmed the effectiveness of 16S rRNA sequencing in identifying native *B. subtilis* strains with biocontrol properties. The authors stressed the importance of combining molecular tools with functional assays to validate species identity and application potential.

CONCLUSIONS

Study while investigating chicken feather degradation under laboratory conditions using termite originated *Bacillus* species: *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus thuringiensis* as a bacterial species. This bacteria species is capable of producing protease degrades the keratin present in chicken feather. The study highlighted that aerobic condition with pH 7 ± 0.5 able to degrade chicken feather as early as 5 days with consortium of three isolates to as maximum of 13 days with *Bacillus thuringiensis* treatment, while median range of degradation was recorded as 8 days by *Bacillus subtilis*. The study therefore concluded to use aerobic condition with pH 7 ± 0.5 with feather degrading media using consortium of *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus thuringiensis* in 1:1:1 proportion with total 10% of inoculum start-up. Therefore, use of consortium is recommended as a feather degrader to achieve degradation by the 5th day.

Conflict of Interest: The authors declare no conflict of interest in relation to this research.

Data Availability Statement: Not applicable.

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