



Isolation, identification, and molecular characterization of potential keratinolytic fungus sp. from Southern Assam: relevance to poultry wastes and its biological management

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Abstract

Feather waste is a highly prevalent form of keratinous waste that is generated by the poultry industry. The global daily production of feather waste has been shown to approach 5 million tons, typically being disposed of through methods such as dumping, landfilling, or incineration which contribute significantly to environmental pollutions. The proper management of these keratinous wastes is crucial to avoid environmental contamination. The study was carried out to isolate the keratinolytic fungi from the poultry disposal sites of different region of North-East India to evaluate its potential in bioremediation of the feathers wastes. Out of 12 fungal strains isolated from the sites, the fungus showing the highest zone of hydrolysis on both the skim milk and keratin agar medium was selected for the study and the molecular identification of the isolate was performed through DNA sequence analysis by amplifying the internal transcribed spacer (ITS) region. The sequence results showed higher similarity (above 95%) with *Aspergillus* spp. and was named *Aspergillus* sp. Iro-1. The strain was further analyzed for its feather degrading potential which was performed in submerged conditions under optimized conditions. The study showed that the strain could effectively degrade the feathers validated through weight loss method, and the structural deformations in the feathers were visualized through scanning electron microscopy (SEM). *Aspergillus* sp. Iro-1 was obtained from the southern region of Assam. It would be of great importance as the implementation of this sp. can help in the bioremediation of feathers wastes in this region. This is the first study of identification of feather degrading fungus from southern part of Assam (Barak).

Keywords *Aspergillus* sp. · Feather bioremediation · Keratinase · Poultry waste · Scanning electron microscope

Introduction

Keratin sources such as feather, horn, nails, and hair, are prevalent in nature as wastes. These keratin wastes are recalcitrant due to the occurrence of strong disulfide bridges, hydrogen bonds, and hydrophobic interactions. Keratin can

be categorized into two distinct types, namely α -keratin and β -keratin, based on their respective structural integrity. The structural arrangement of alpha-helices and beta-sheets within a keratin protein is accountable for its inherent stability and robust characteristics (Mckittrick et al. 2012; Wang et al. 2016; Li 2021). α -Keratin is predominantly present in hair, wool, and horn. β -Keratin is the primary protein found in the feathers and scales of avian and reptilian species (Qiu et al. 2020). According to statistical data, there has been a consistent upward trend in global poultry meat consumption over the recent years (Bohacz 2022). Due to the ongoing consumption of meat and the concurrent increase in global population, a substantial quantity of generated feathers are being disposed as trash or incorporated into soil through tilling practices. The poultry and local farm generates quite large amount of feathers waste which is composed of around 90% of β -keratin. The generation of these keratin rich wastes

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creates an environmental havoc due to its slow degradation in nature. With the expansion of the poultry industry, there is an increase in the accumulation of poultry residues, which causes air, land, and water pollution. In the context of India, it is estimated that chicken industries generate an annual production of around 350 million tons of keratinous wastes in the form of feathers (El-ghonemy and Ali 2021). Traditional methods of feather degradation such as incineration, land-filling, composting, and mechanical grinding and chemical method using strong acid or alkali solutions are expensive processes and leads to environmental pollution along with the deterioration of amino acid and polypeptide integrity inside the feathers (Zhang et al. 2022). Given the aforementioned circumstances, there arises a necessity for the establishment of an alternate approach to recycle keratin-based waste materials. Hence, the utilization of microorganisms for the breakdown of keratin waste presents a viable option due to their widespread presence in the environment and their ability to produce diverse proteases of significant biotechnological value. Therefore, the microbial approach for keratin biodegradation can be effective as it is environmental friendly and cost-effective method. Microorganism such as fungi plays a crucial role in the degradation of keratin (feather) by producing keratinase, which breaks the numerous rigid bonds in feather's keratin, thereby reducing the solid waste problem. The utilization of microbial keratinase to degrade feathers is a highly effective approach for preserving the nutritional value of amino acids derived from feather decomposition which can be used as animal feed supplements or as organic fertilizers (Călin et al. 2017; Parmar and Trivedi 2021). In addition to their ability to degrade resistant keratin, keratinases are also utilized in other biotechnological fields. The addition of keratinases to animal feed was also associated with increased chicken weight (Odetallah et al. 2003). When keratinases are employed as a substitute for chemicals in the dehairing process, it has been observed that leather generated through keratinases treatment exhibits more integrity and superior quality in comparison to leather treated with chemicals (Ben Elhoul et al. 2021). In addition, it can serve as a biopesticides, effectively eliminating root nematodes that are responsible for generating pest-related diseases (Yue et al. 2011). Recent research has been conducted utilizing keratinases as a transdermal drug delivery agent in conjunction with the drug (Shalaby et al. 2021). Both bacteria and fungi have the ability to secrete significant amounts of keratinases. The enzymatic degradation of feathers is considered to be environmentally advantageous, as it helps to maintain the nutritional value of the resulting byproduct. In contrast, the physical and chemical treatments employed provide amino acids of lower quality (Qiu et al. 2020).

Fungi possess the capacity to proliferate on cost-effective substrates and excrete substantial quantities of diverse

enzymes into the surrounding culture media (Anitha and Palanivelu 2013). Filamentous fungus, such as *Aspergillus*, has the ability to thrive on cost-effective substrates, specifically solid agro-industry wastes, and possess the capacity to release varieties of enzymes (Hernández-Martínez et al. 2011). Fungi, being widely distributed in the natural environment, have the ability to synthesize extracellular proteases, including keratinases, which have the capacity to enzymatically degrade the resilient and resistant keratin substrate. Several species of fungi that are capable of degrading keratin have been documented in the literature. Some of the keratin-degrading fungi include *Aspergillus niger*, *Trichoderma harzianum*, *Aphanoascus keratinophilus*, *Chrysosporium tropicum*, *Trichophyton ajelloi*, and *Aspergillus flavus*, etc. (Maria et al. 2013; Bagewadi et al. 2018; Bohacz et al. 2020; Masood et al. 2021; Bohacz 2022).

The present study focuses on the isolation of fungal strains from soil samples collected from various poultry disposal sites situated in distinct regions of the North-Eastern region of Assam. The isolate was chosen based on its ability to produce a hydrolytic zone on the skim milk and keratin agar media. The selected isolate was carried forward to investigate its potential for the remediation of keratin (feather) wastes generated by the poultry industry. This article presents an introductory examination of a fungal species capable of degrading feathers, which has been isolated from the North-Eastern region of India.

Materials and methods

Collection of soil sample

The soil samples included in this study were obtained by collecting them from areas where poultry wastes is disposed off in various regions of North-Eastern region of India. Approximately 5 g of soil were collected from a depth of 2–3 cm at areas designated for the disposal of poultry waste.

Feather collection and pretreatment

The chicken feathers were obtained from areas where poultry waste was disposed. They were subjected to a thorough washing process using distilled water and afterwards dried under sunlight. The dried feathers were sterilized using chloroform: methanol at 1:1 (v/v) for 1 h and was washed twice thoroughly with distilled water and dried at 60 °C and autoclaved. Finally, the feathers were chopped and stored at room temperature to use as keratin substrate in fermentation process (Yusuf et al. 2020).

Extraction of keratin from feathers

The preparation of keratin powder was conducted following the methodology outlined by Mazotto et al. (2011). A total of 5 g of autoclaved feathers were subjected to a heating process lasting 2 h, in 250 ml of dimethyl sulfoxide (DMSO). To precipitate keratin, a volume of 500 ml of acetone is added, and the resulting mixture is stored at a temperature of $-20\text{ }^{\circ}\text{C}$ for duration of 24 to 48 h. The keratin was obtained using the process of centrifugation (10,000 rpm for 15 min). The obtained precipitate was subjected to two rounds of washing with distilled water, followed by drying at a temperature of $40\text{ }^{\circ}\text{C}$. This dried precipitate was subsequently utilized for secondary screening purposes, as well as for the assessment of its keratinolytic activity (Mazotto et al. 2011).

Isolation of keratinolytic fungi

The soil samples were obtained from the location where poultry farms deposit feathers, with the purpose of isolating fungi. The locations encompass several regions of North-East India. The soil samples, weighing approximately 5 g each, were carefully gathered and placed in sterile, airtight polybags. The fungi were isolated through the implementation of the serial dilution and plating techniques on a keratin agar medium (KAM). A quantity of 1 g of soil samples, along with the traces of feather obtained from chicken disposal sites, was combined with 10 ml of sterile distilled water and mixed extensively. Serial dilution was performed up to a dilution factor of 10^{-10} . In this experiment, 100 μl of the diluted soil sample was inoculated into the keratin agar medium. The composition of the medium included 2.5 g/L of keratin, 0.25 g/L of MgSO_4 , 0.115 g/L of KH_2PO_4 , 0.25 g/L of K_2HPO_4 , and 5 g/L of agar. To prevent bacterial growth in the keratin medium, 0.1% of streptomycin was added. The fungal specimens were gathered during a 4-day incubation period at a temperature of $37\text{ }^{\circ}\text{C}$. The specimens were subjected to a meticulous process of isolation and thereafter kept in SDA (Sabouraud dextrose agar) at a temperature of $-4\text{ }^{\circ}\text{C}$. The composition of the medium (g/250 ml) is dextrose-10, peptone-2.5, and agar-5 (Mini et al. 2012).

Screening of keratinolytic fungi

The quantification of the isolates' keratinase activity was assessed using primary and secondary screening methods on skim milk agar and keratin agar medium. Skim milk agar plates were constructed for the main screening of proteolytic activity. The composition of the plates included 5 g per liter of peptone, 3 g per liter of yeast extract, 100 ml per liter of sterile skimmed milk, and 15 g per liter of agar. The isolates were inoculated on agar plates with keratin agar (KA) with

the following concentrations (g/l): NaCl (0.5), KH_2PO_4 (0.7), K_2HPO_4 (1.4), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1), keratin powder (10), agar (15), and adjusted to a pH of 7.5. The plates were subjected to incubation at a temperature of $37\text{ }^{\circ}\text{C}$ for duration of 4 days (Sutoyo et al. 2019). The keratinolytic activity of each isolate was assessed by calculating its clear zone index, which was derived by comparing the diameter of the colony to the diameter of the clear zone.

Identification of selected isolate

Morphological features

The fungus was chosen based on both primary and secondary screening processes. The fungus with the greatest hydrolytic zone or protease activity was subsequently selected for further investigation. The fungal isolates were subjected to microscopic examination with the lactophenol blue staining technique. The investigation focused on the examination of morphological structures to ascertain the fungal strains.

Molecular identification of the selected isolate

The process of molecular identification of fungi was conducted by employing ITS sequencing, a technique known for its conservation across many fungal species. In addition to the investigation of phenotypic features, the molecular identification was conducted using PCR analysis and nucleotide sequencing. Initially, the fungi were cultivated in flasks containing Sabouraud dextrose broth and subjected to incubation at a temperature of $25\text{ }^{\circ}\text{C}$ for duration of several days, employing a shaker rotator. The pure culture was then sent to Eurofins, Bangalore for sequence analysis. The ITS1–5.8S–ITS 2 rDNA was amplified using ITS1 (3'-TCC-GTAGGT- GAA-CCT-GCG-G-5') and ITS4 (3'-TCC-TCC-GCTTAT- TGA-TAT-GC-5'), as forward and reverse primers as described by White et al. (1990). The sequencing findings were analyzed using the web-based basic local alignment search tool (BLAST) application, available at the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/BLAST>). The obtained data were then compared with the NCBI/Gene bank database. The Maximum Likelihood approach was employed to infer the evolutionary history, utilizing the Tamura–Nei model (Tamura and Nei 1993). Evolutionary studies were performed with the Mega-X software, following the methodology outlined by Kumar et al. in 2016 (Kumar et al. 2016).

Biomass production of the *Aspergillus* sp. Iro-1 under submerged conditions

To investigate microbial growth and substrate breakdown, the chosen microorganisms were cultivated in 250 ml Erlenmeyer flasks, each containing 100 ml of media and 0.1 g of

feather keratin. The flasks were inoculated with a suspension of fungus that was 5 days old and had a concentration of 2×10^6 spores per milliliter. A basal solution was utilized in the experiment, including the following composition expressed in grams per liter (g/l): the experimental solution consisted of the following components: 0.1 potassium dihydrogen phosphate (KH_2PO_4), 0.01 calcium chloride (CaCl_2), 0.1 iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), and 0.005 zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). The pH of the solution was measured to be 7.5. The experimental control group included just of the basal mineral solution and keratin substrate, devoid of any microorganisms (Kumawat et al. 2017). The fungal cultures underwent filtration using Whatman filter paper No. 1. The filtrate was utilized as a source of unrefined enzymes for the keratinase assay.

Keratinase assay

The fungal strain *Aspergillus* sp. Iro-1 (Accession no. OQO80047) was cultivated on a potato dextrose agar medium at a temperature of 30 °C. The keratinolytic activity was conducted using the methodology outlined by Gradišar et al. (2000), with feather keratin as the substrate. The reaction mixture consisted of 1 ml of a 28 mM tris–HCl buffer with a pH of 7.5, 100 µl of crude enzyme, and 5 mg of keratin powder. The incubation process is conducted at a temperature of 37 °C for duration of 1 h, while ensuring continuous agitation at a speed of 160 rpm. A volume of 0.5 ml of trichloroacetic acid (TCA) solution with a concentration of 10% was introduced to halt the enzymatic reaction. The mixture was afterwards stored at a temperature of 4 °C for duration of 30 min. TCA was introduced prior to incubation to establish control. Following centrifugation with a force of 14,000 g for a duration of 15 min, the absorbance of the supernatant fluid was quantified at a wavelength of 280 nm. The unit (U) of keratinase was operationally defined as a 0.01 rise in absorbance under the specified assay conditions (Gradišar et al. 2000).

$$U = 4 \times n \times A_{280} / (0.01 \times 10)$$

where n is the dilution factor; 4 is the final reaction volume (ml); 10 is the incubation time.

Optimization of physical parameters for fungal keratinase production

The optimization of pH and temperature for keratinases production and feather degradation was performed by one variable at a time approach (OVAT). The fungal spores inoculated in the minimal basal media at the pH (6–10) and the temperature (25 °C to 50 °C) for 7 days were examined (Anbu et al. 2008).

Biodegradation of feathers

The determination of feather degradation by *Aspergillus* sp. Iro-1 was conducted using the weight loss method. The feathers that had been pre-treated were afterwards utilized in the fermentation broth. The feather degradation analysis was conducted using the methodology provided by Tarun et al. (2017), with slight modifications (Kumawat et al. 2017) in basal salt medium containing (g/l) NaCl, 0.5; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1; CaCl_2 , 0.06; KH_2PO_4 , 0.5; and K_2HPO_4 , 0.5; feather, 1.0. The degradation rate was analyzed after every alternative 5 days till day 30. The chicken feathers were obtained from the fermentation broth and subjected to filtration using Whatman No. 1 filter paper. Subsequently, they were rinsed twice with distilled water, dried at a temperature of 50 °C, and their weight was measured. The quantification of keratin biodegradation was accomplished by calculating the disparity in residual dry weights between a treated sample and its corresponding control (keratin devoid of fungal inoculation) after a period of 30 days. The calculation of feather deterioration percentage is determined by

$$\text{Percent feather degradation \%} = \frac{W - F}{W} \times 100$$

where F = feather weight after fermentation, W = feather weight before fermentation.

Scanning electron microscopy (SEM)

The utilization of scanning electron microscopy (SEM) is employed for the examination of the microscopic degradation pattern observed in chicken feathers. The feather incubated with the fungal strain *Aspergillus* sp. Iro-1 was recovered from the culture media after 21 days. The SEM analysis for other keratin substrates such as horse hair, (Călin et al. 2017) human hair, (Gurung et al. 2018) was performed at day 21. The deteriorated feathers were washed twice with distilled water, followed by dehydration using acetone, and then air-drying. Subsequently, the specimens were affixed to the supports utilizing double-sided carbon tape. The specimens were dispatched to North Eastern Hill University (NEHU), located in Shillong, for scanning electron microscopy (SEM) investigation.

Result

Collection of soil samples

The keratinolytic fungal strains were isolated from soil samples collected from the different poultry sites around

North-East India (Fig. 1). The sample sites include as follows:

S1- College road, Silchar (District: Cachar, State: Assam, Coordinate: 24°48'59.0"N/92°47'19.9"E).

S2- Irongmara, Silchar (District: Cachar, State: Assam, Coordinate: 24°41'20.4"N/92°44'30.5"E).

S3- Dharmanagar (District: North Tripura, State: Tripura, Coordinate: 24°22'41.6"N/92°09'25.6"E).

S4- Buragoan, Guwahati (District: Kamrup, State- Assam, Coordinate-26°06'37.6"N/92°42'26.7"E).

S5- Mahur (District: Dima Hasao, State: Assam, Coordinate: 25°09'29.7"N/93°07'22.9"E).

S6- Hawaipur (District: West Karbi Anglong, State: Assam, Coordinate: 25°51'32.1"N/92°58'05.3"E).

S7- Borapani (District: East Khasi hills, State: Meghalaya, Coordinate: 25°40'36.8"N/91°55'39.7"E).

S8- Dimapur (District: Dimapur, State: Nagaland, Coordinates: 25°54'49.3"N/93°43'21.4"E).

S9- Haflong (District: Dima Hasao, State: Assam, Coordinate: 25°07'47.2"N/93°01'26.9"E).

Screening of isolates for protease activity

Upon the screening of fungi on skim milk agar medium and keratin agar medium, the isolate showing higher zone of hydrolysis was the media that was screened and named as *Aspergillus* sp. Iro-1 (Fig. 2). Then the selected isolate was carried forward for further degradation studies. Keratinolytic



Fig. 1 Different sites of the feather dumping sites in the North-Eastern region, India (S1-S9)

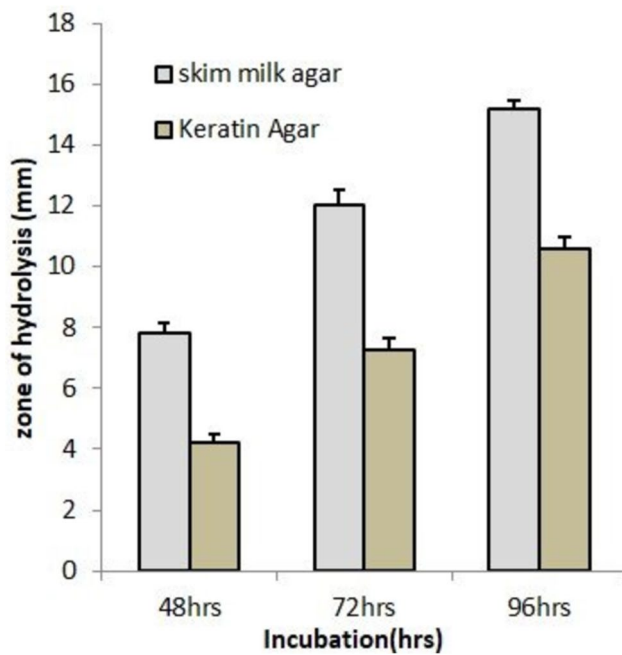


Fig. 2 Hydrolysis zone diameters of *Aspergillus* sp. Iro-1 strains on skim agar plate and keratin agar. The values represent mean \pm S.E.M of three independent experiments

activity of fungi was detected as clear zones around the colony after incubation for up to 96 h at room temperature.

Molecular identification

The sequences produced from the process of sequencing were subjected to analysis. The contig was generated by aligning the forward and reverse sequences, and then the resulting sequences were submitted to the NCBI BLAST database for analysis. A BLAST search was conducted on the sequences to compare them with the NCBI-GenBank database. The findings indicated that the isolate exhibited a greater proportion of identification with *Aspergillus flavus* (96.84%) and *Aspergillus oryzae* (96.54%). The isolate was named as *Aspergillus* sp. Iro-1 (Accession no. OQO80047) as it possessed greater similarities with *Aspergillus* species. The construction of the phylogenetic tree (Fig. 3) was performed with the Mega-X software. The phylogenetic analysis of the organism indicated a greater degree of similarity with *Aspergillus flavus* and *Aspergillus oryzae*.

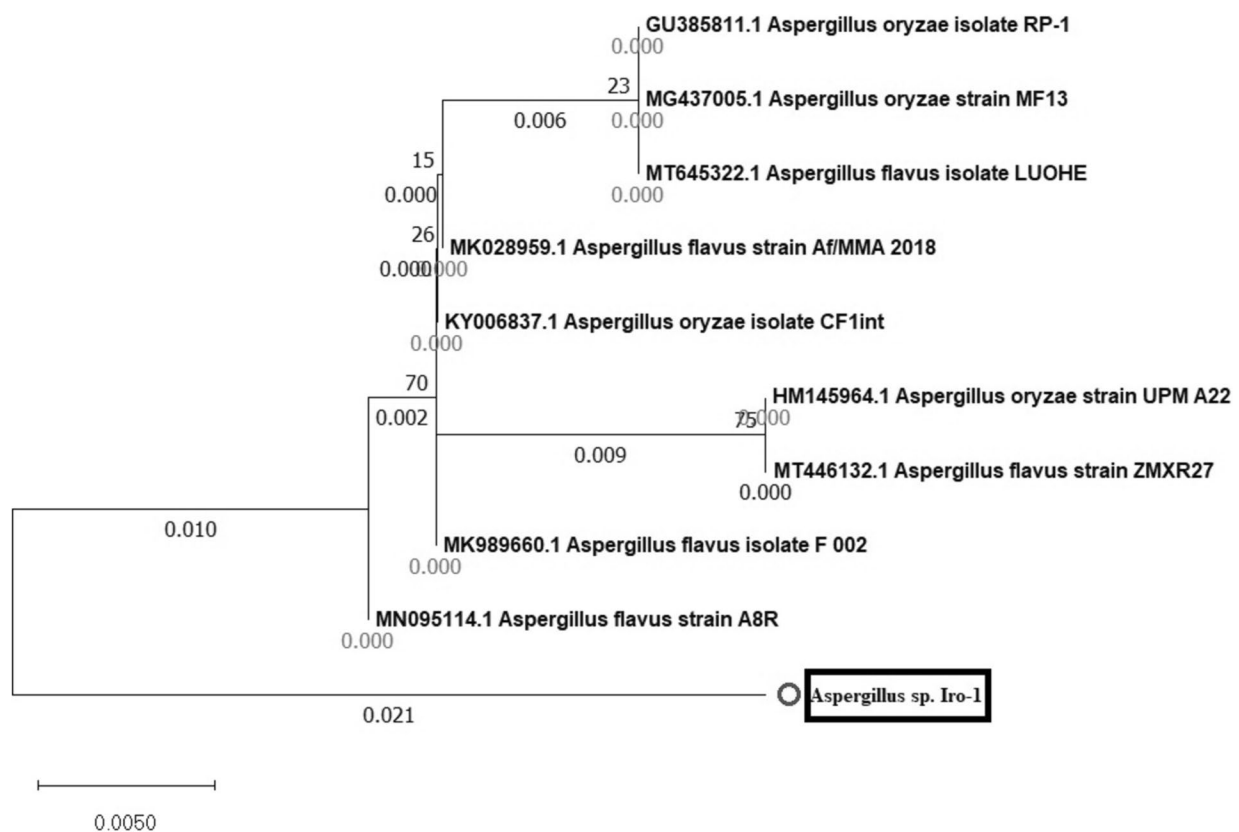


Fig. 3 Phylogenetic tree analysis of the *Aspergillus* sp. Iro-1 with the related strains

Optimization of conditions for keratinase production

The optimization of conditions for keratinases production was found to show maximum keratinase production between pH (7–8). The optimized pH was found to be at pH-8.0 after which the keratinase production tends to decrease. The optimal temperature was at 30 °C (Fig. 4).

Feather degradation

The measurement of feather degradation was conducted by assessing the state of the feathers following a fermentation period of 5, 10, 15, 20, 25, and 30 days of incubation. An increase in the percentage of feather deterioration was seen as the duration increased. The data shown in Fig. 5 indicate that around 49.5% of the feathers had degeneration by the 30th day. The texture of the control feathers and the feathers treated with *Aspergillus* sp. Iro-1 on the 30th day is depicted in Fig. 6. The degradation of barbs, barbules, and rachis was observed on the 30th day. The initial weight of the control flask was 1 g, it was kept without any fungal inoculation and its weight remained unaffected throughout the incubation days.

SEM analysis

The investigation using a scanning electron microscope (SEM) was conducted to verify the destruction of feathers by the fungus *Aspergillus* sp. Iro-1. The micrographs illustrate

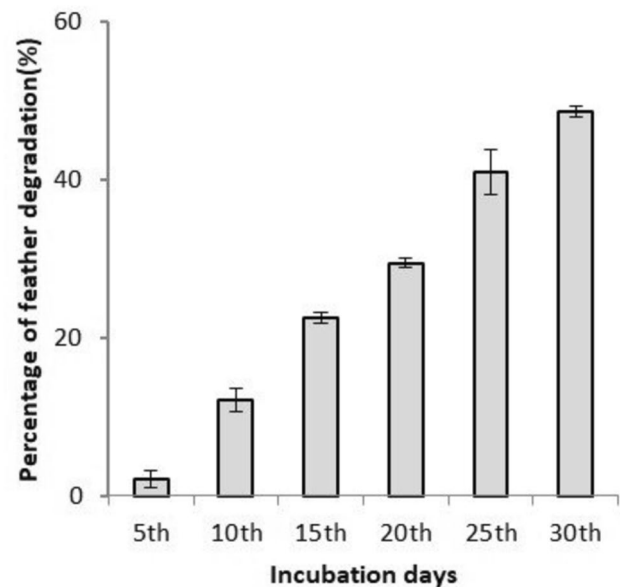


Fig. 5 The percentage of feather degradation with the increase in incubation days. The values represent mean \pm S.E.M ($n=2$)

the degradation of both the rachis and barbs of the feather, indicating the progressive deterioration and weakening of its structural integrity. Figure 7A–C demonstrates that the feathers that were not subjected to any treatment maintained their structural integrity. However, the feathers that were treated with the isolate exhibited degradation in both barbs and barbules (Fig. 7D and F), as well as corrosion in the rachis (Fig. 7E).

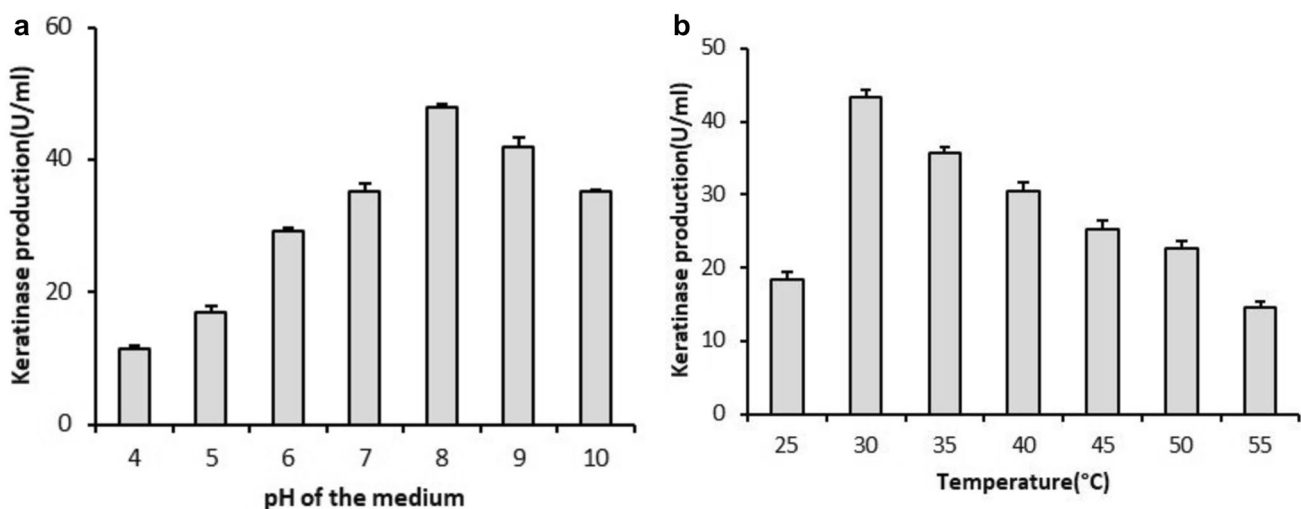


Fig. 4 The keratinase production at different pH (A) and temperature (B); it was found that the keratinase production was higher at alkaline pH and temperature of 30 °C. The values represent mean \pm S.E.M of three independent experiments



Fig. 6 Feather texture at 30th day. **A** Control flask (without fungus). **B** Treated with *Aspergillus* sp. Iro-1

Discussion

Keratinolytic fungi possess the capability to inhabit various keratin-based substrates, where they enzymatically degrade and utilize these substrates as carbon and nitrogen sources. The keratinolytic property of microorganism was determined through their ability of feather degradation and higher yield of keratinase production. The current study is the first report for the isolation and identification of keratinolytic fungus from the poultry dump sites from North-Eastern region of India.

The fungal strains isolated from the sites were screened through primary and secondary screening processes. The strain showing higher zone of hydrolysis on both skim milk agar medium and keratin agar medium was selected for the biodegradation of feather. The strain showing higher hydrolytic zone on both the media was identified (molecular identification) and named as *Aspergillus* sp. Iro-1 (Accession number OQO80047). The potential fungal strain was isolated from the poultry waste disposal sites of North East, India (Assam). Similar study was carried out to isolate various other keratinolytic fungi from different regions of India which included *Scopulariopsis brevicaulis* from poultry farm soil at Namakkal (Anbu et al. 2005), *Trichophyton* sp. HA-2 from feather dumping soil in India (Anbu et al. 2008), *Chrysosporium queenslandicum* TKKASb from

poultry farm house soil of Rajasthan (Kumar et al. 2017), *Aspergillus parasiticus* from poultry soil (India) (Anitha and Palanivelu 2013). Similar study was carried out by Devi et al. 2021, where keratinolytic fungi were isolated from Loktak Lake, Manipur (India). The keratinolytic fungal isolates were found to be under the following genera: to six genera: *Trichoderma harzianum*, *Curvularia lunata*, *Curvularia affinis*, *Poitrasia circinans*, *Fusarium oxysporum*, *Chrysosporium mephiticum*, *Chrysosporium lucknowense*, *Westerdykella dispersa* (Agrawal et al. 2021).

The screening of the keratinolytic fungi was carried out through primary (skim milk agar) and secondary screening (keratin agar medium) and the isolate showing higher zone of hydrolysis was further carried forward for molecular identification. Similar study for the screening of other keratinolytic fungi was reported for the fungi, *Aspergillus niger* (Maria et al. 2013). The keratinase assay was performed as described by Mazotto et al. 2011 using keratin (feather) powder as substrate and the absorbance was calculated at 280 nm and the increase in 0.01 in absorbance was calculated to be one unit of keratinase enzyme (Mazotto et al. 2011). The similar keratinase assay was reported for microorganisms such as *Scopulariopsis brevicaulis* (Anbu et al. 2005), *Trichophyton* sp. HA-2 (Anbu et al. 2008), *Candida parapsilosis* (Duarte et al. 2011), *Bacillus subtilis* SLC (Cedrola et al. 2012), and *Bacillus aerius* NSMk2 (Bhari et al. 2021).

The optimization keratinase production from the selected isolate was optimized at different conditions of pH and temperature. The optimum temperature for keratinase production was found to be at pH-8. The optimum pH of 8.0 was similar to the keratinase from *Trichophyton* sp. HA-2 (Anbu et al. 2008), *Aspergillus flavus* strain K-03 (Kim 2010), *Pseudomonas* sp. LM19 (Mohamad et al. 2017), *Bacillus licheniformis* ALW1 (Abdel-fattah et al. 2018), *Bacillus cereus* HD1 (Yahaya et al. 2022). The study suggests that the keratinase production is inducible process as it gets secreted in presence of the keratin rich substrate. The possible reason for higher keratinase production at alkaline pH could be the properties of keratinase enzyme which tend to be stable at alkaline pH, thus aid in feather degradation as well. The optimized temperature for the *Aspergillus* sp. iro-1 was found to be at 30 °C and the similar result was found for the keratinase production for *Aspergillus* sp. DHE7 (Elghonemy and Ali 2017).

The degradation study also validated the feather degrading properties of the fungus, *Aspergillus* sp. Iro-1 which was investigated through weight loss method which was further morphological validated through SEM analysis. It was observed that around 49.5% of the feathers underwent degradation by the 30th day, accompanied by minor corrosion in the rachis. The experiment revealed that on the 42th day, the feather had undergone complete degradation. The

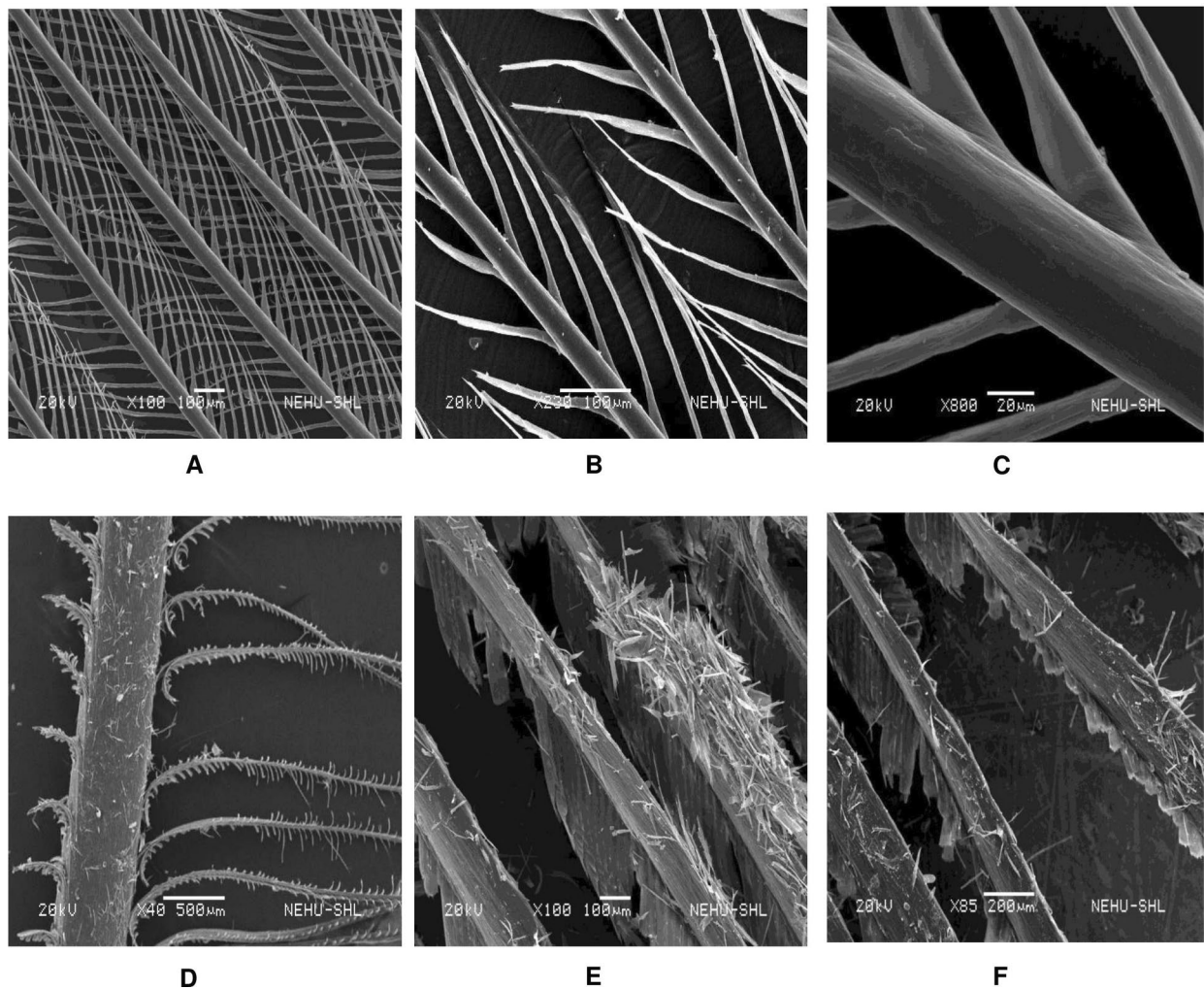


Fig. 7 Scanning electron microscope image of control (A–C) and treated feather (D–F) by *Aspergillus* sp. Iro-1

findings were corroborated by the experiment conducted by Muhsin et al. (2001), which revealed that the degradation of chicken feathers (32%) occurred when they were incubated with *Aspergillus flavus* in a liquid culture medium for a period of 3 weeks, utilizing feathers as the substrate (Muhsin and Hadi 2002). Masood et al. 2021 reported that 74% of feather degradation was observed at 30th day of incubation when the feather was treated with *Aspergillus flavus* (Masood et al. 2021). The other study supporting our data demonstrated a $46.40 \pm 2.50\%$ degradation of feathers by the fungus *Chrysosporium queenslandicum* after 24 days of incubations and the morphological changes in the feather were validated through SEM analysis (Kumawat et al. 2017). Similarly fungal species *Aphanoascus keratinophilus* and *Chrysosporium tropicum* strains after 8th weeks of incubation showed around 60–75% and 50–70% degradation of feather, respectively (Bohacz et al. 2020). The feather degradation study was also carried out by *Trichophyton ajelloi* and it was found that 58% of feather degradation was

observed after 42 days of fungal inoculation (Bohacz 2022). In congruence to the above discussed studies, our study is the first to report the isolation of keratinolytic fungi from the poultry dumped sites covering the North-Eastern region of India for their potential in degradation of poultry feather at optimized conditions.

Conclusion

The proliferation of the poultry industry contributes to the accumulation of feathers waste, which leads to solid waste problem. The management strategies utilized for keratinous substrates, including landfilling, burning, steam pressure boiling, and strong alkali or acid hydrolysis, not only result in adverse environmental consequences but also necessitate substantial energy consumption. Moreover, these methodologies result in the deterioration of specific essential amino acids. Hence, it is imperative to choose effective strategies

for the appropriate management of these keratinous wastes. The microbial degradation of keratinous substances through keratinases represents a cost-effective and environmentally responsible method for efficiently managing keratinous wastes. This technique ensures environmental safety, waste management, and the creation of valuable resources such as peptides and amino acids. The present study for the first time demonstrated the isolation and identification of *Aspergillus* sp. Iro-1 from the poultry dumping sites around North-East India and the potential of this strain in degrading the keratinous substrates. The findings of this study could be of great importance as this wild fungus *Aspergillus* sp. Iro-1 could effectively degrade the feathers and can be used in the management of feathers wastes, thereby minimizing the risk of environmental pollution.

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Author contributions IG: conceptualization, experimentation, data analysis, data interpretation, data validation and writing the original draft, RR: writing and editing, BCP: writing and editing, DK: editing and reviewing, PP: conceptualization of the work, SK: conceptualization of the work; AB: conceptualization, editing and reviewing.

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Data availability All datasets generated or analyzed during this study are included in the manuscript.

Declarations

Conflict of interest All authors disclosed no conflict of interest.

Ethics approval Not applicable.

Consent to publish All the work reported in the manuscript is original and has not been submitted in any other journal. The copyright of the manuscript is authorized to the journal by the authors upon acceptance for publication.

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