Research article

Exiguobacterium acetylicum transformed poultry feathers into amino acids through an extracellular secretion of keratinolytic enzymes

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Abstract: The transition from a traditional linear economy to a circular model aims to create a more sustainable future by reducing the adverse effects of agro-waste on the environment. The present study evaluated the metabolic diversity of bacterial isolates from municipal dumpsites for keratinase production and poultry feather valorization. The bacterium with the highest keratinolytic activity was identified through 16S ribosomal ribonucleic acid (rRNA) gene sequencing. The exo-keratinase production by the bacterium was optimized, and the feather hydrolysate obtained from the fermentation process was analyzed for amino acids. Among the twelve bacteria isolated from the dumpsite's sample, three showed significant feather degradation and keratinase production of 89% (744.5 ± 9.19 U/mL), 58% (269 ± 15.55 U/mL), and 46% (195 ± 7.07 U/mL) for SSB-03, SSB-02, and HSB-02, respectively. Analysis of the 16S rRNA gene sequence revealed that SSB-03 has high sequence homology with *Exiguobacterium acetylicum*, and thus, it was identified as *Exiguobacterium acetylicum* FHBD (accession number MW165834). Strain FHBD fermentation medium exhibited the maximum keratinase activity (2934.54 ± 38.56 U/mL) and sulfhydryl group content (3.09 ± 0.02 mM) at 72 h under optimal process conditions of pH 5.0, temperature (35 °C), inoculum size (2% v/v), and feather (15 g/L). Amino acid analysis of the feather hydrolysate showed significant levels of glutamic acid, aspartic acid, glycine, arginine, serine, and proline, with respective concentrations of 1.58, 1.34, 1.29, 1.20, 1.12, and 0.93 (g/100 g of sample). The study's findings emphasize the potential of *E. acetylicum* FHBD in poultry feather valorization and keratinase production.

Abbreviations: MSM: Minimal salt media; GCF: Ground chicken feather; PCR: Polymerase chain reaction; NCBI: National Centre for Biotechnology Information; BLAST: Basic local alignment search tool

1. Introduction

Advocating for the transition from a linear economy to a circular one, which prioritizes the use of renewable bioresources for green energy and biochemicals, can help address the global dependency on finite fossil-based resources. This shift can contribute to a more sustainable future by reducing the negative impact of waste handling [1]. However, the increasing waste stream from agro-industrial sectors could potentially overwhelm the Earth's biophysical capacity due to population growth. Therefore, it is important to explore alternatives to incorporating renewable waste biomass into the economy to support the concept of circularity and promote sustainable development [2].

The poultry industry plays a significant role in global food security by providing nutritious meats and meat products [3]. However, the processing of poultry meat generates variable by-products, including keratinous feathers, which make up a significant portion of the waste stream. In order to stimulate a sustainable and healthy agroecosystem, it is crucial to integrate efficient poultry feather recycling into the poultry production value chain. Avian feathers display high mechanical and structural stability due to their unique architectural disposition of interactive linkages, which hold the biomass together [4]. This results in super-durability and high resistance to mechanical damage or biological attack [5]. Researchers have explored various processing methods, such as pressure cooking, mechanical crushing, and chemical hydrolysis, to salvage the high crude protein content of keratinous feathers [6]. The limitations associated with existing technologies for processing keratinous poultry feathers have prompted the search for more sustainable alternatives. Microbial technology, which is both eco-friendly and cost-effective, has been identified as a promising approach for the valorization of keratin-rich waste [7].

Environmental stressors can influence prokaryotes' ability to use a variety of substrates in their environment. Stress conditions cause changes in gene expression, including the upregulation of genes in biosynthetic pathways as part of the bacterial stress response mechanism [8]. In addition to keratinophilic fungi that inhabit keratin-rich areas and secrete keratinase as a means of causing disease [9], bacteria have been widely reported to break down keratinous biomass through the expression of a robust keratinolytic system [10]. This green technological approach has gained significant traction since the isolation and characterization of *Bacillus licheniformis* PWD-1 from a poultry facility [11].

To date, various strains of Gram-positive and Gram-negative bacteria with keratinolytic potential have been isolated from different environments. However, there is a lack of uniformity in the ability of these bacterial strains to degrade keratinous biomass. For example, *Bacillus cereus* HD1 [12], *Stenotrophomonas maltophilia* BBE11-1 [13], *Pseudomonas geniculata* H10 [14], *Chryseobacterium* sp. RBT [15], *Kocuria rhizophila* p3-3 [16], and *Streptomyces netropsis* A-ICA [17] were able to degrade feathers by $57 \pm 2\%$, 22.8%, $82.3 \pm 3.5\%$, 83%, 52%, and $84 \pm 2\%$, after 168, 96, 36, 48, 96, and 120 h, respectively.

Therefore, several bacterial strains are being studied for their ability to effectively convert keratinous biomass into valuable products. The efficient decomposition of poultry feathers by wild bacterial species is promising for the sustainable production of protein hydrolysates, which contain significant amounts of bioactive peptides, amino acids, and non-protein nitrogen [18,19]. Protein hydrolysates generated through microbial fermentation of poultry feathers have been utilized as soil fertility enhancers due to their slow nitrogen-releasing power, plant growth hormone content, and microbial soil-nutrient recycling potential [20].

Despite the potential of municipal dumpsites as a source of novel bacterial strains with diverse metabolic activity, they remain underexplored. The complex interactions among microbial populations in this ecological niche and their involvement in nutrient recycling highlight the importance of microbial technology in achieving a regenerative and circular economy [21]. Production of hydrolytic enzymes ensures that bacteria extract relevant nutrients from both soluble and insoluble substrates for homeostasis. Therefore, this study explored the bacterial diversity of local municipal dumpsites for feather-degrading bacteria. A potent isolate, identified as *E. acetylicum* FHBD, was evaluated for its ability to secrete keratinase in a cheaply formulated medium, and the fermentation process conditions were optimized. The protein hydrolysate generated through bacterial digestion of chicken feathers was analyzed for various amino acids.

2. Materials and methods

2.1. Sample collection and bacteria isolation

Soil samples were collected from the municipal dumpsites in Alice environment (32.7901° S, 26.8330° E) where variable biomaterials have decomposed. The samples were taken to the laboratory for further analysis. Approximately 1 g of soil samples was inoculated into a 250 mL Erlenmeyer flask containing 99 mL of minimal salt medium (MSM) with 10 g/L of ground chicken feathers (GCF) as the only carbon source and incubated with a constant agitation (130 rpm) for 7 days at 30 °C. The MSM composition was as follows (g/L): K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl_2$, 0.2; $CaCl_2$, 0.22; NH_4Cl, 0.5 [22]. The isolation medium (100 μL) was spread on minimal salt agar after appropriate dilution of the medium. The agar plate contained similar compositions as the MSM, with additions of 15 g/L bacteriological agar and 50 μg/L of nystatin. The agar plates were incubated for 72 h and monitored for bacterial growth. Different colonies were picked from the bacterial culture based on their morphological characteristics and subcultured on freshly prepared plates. Pure bacterial isolates were preserved on GCF agar slant at 4 °C.

2.2. Inocula preparation

From the slant, a loopful of pure isolates was streaked on the GCF agar plates and incubated for 24 h at 30 °C. Bacterial cultures were further standardized on 0.5 McFarland bases, reading 0.1 absorbance at 600 nm. These inocula were used for subsequent evaluation and fermentation processes.

2.3. Screening the isolates for protease activity

The isolates were subjected to skimmed milk hydrolysis evaluation as reported previously [23]. Skimmed milk agar plates were prepared using MSM, skimmed milk (10 g/L), and bacteriological agar (15 g/L). Freshly standardized inoculum (10 µL) of each bacterial culture was placed onto the skimmed milk plates and incubated at 30 °C for 24 h. Subsequently, the plates were observed for clearance zones around the isolate, and the plates showing halo zones were considered protease-producing bacterial isolates.

2.4. Evaluation of intact chicken feathers degradation by proteolytic bacteria

The proteolytic bacteria were evaluated for chicken feather degradation capability in MSM (without NH$_4$Cl) supplemented with intact chicken feathers (10 g/L) as the only carbon and nitrogen source. The Erlenmeyer flasks (250 mL) containing 100 mL of working volume were incubated for 72 h in a rotary shaker (130 rpm) at 30 °C. The degree of chicken feathers' hydrolysis was assessed in the flasks showing feathers degradation by visual inspection and determination of percentage feather hydrolysis [24]. Extracellular keratinase production was determined using cell-free filtrates extracted by centrifugation. The isolates that showed significant degradation of chicken feathers were considered potent keratin degraders and were selected for further studies.

2.5. Determination of keratinase activity using cell-free extracts

Keratinase activity was determined using the method described by Nnolim et al. [25]. Keratin-azure (Sigma-Aldrich, St. Louis, MO, USA) was used as an assay substrate. The keratin-azure powder (10 g/L) was suspended in 0.1 M Tris-HCl buffer, pH 7.5. The assay vial contained 0.5 mL of keratin-azure solution and 0.5 mL of crude enzyme. The tube was incubated for 30 min at 37 °C. After incubation, the unutilized substrates were removed by centrifugation at 15,000 rpm for 10 min. Azo dye released from the keratin-azure was determined at 595 nm using a 96-well plate reader (SYNERGYMx, BioTek, USA). One unit of keratinase activity was defined as the amount of enzyme causing an increase of 0.01 in absorbance at 595 nm per minute under the assay protocol described.

2.6. Protein content and sulfhydryl group quantitation

Bovine serum albumin was used as a reference protein to quantify the crude extract's protein concentration as reported by the method of Bradford [26]. Sulfhydryl groups' concentration in the extract was determined according to Ellman's method [27].

2.7. Molecular identification of the promising bacterial isolates

The colonies of the potent keratinolytic isolates were harvested after 18 h of incubation and the deoxyribonucleic acid (DNA) extraction was carried out using the Zymo Research DNA isolation kit (ZR Fungal/Bacterial DNA Kit^{TM}). The DNA template extracted was used to amplify the target 16S rRNA gene sequence using polymerase chain reaction (PCR) under standard thermal cycling

conditions and primer set 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-CGGTTACCTTGTTACGACTT-3') as forward and reverse primers, respectively [28]. PCR was performed using Ready 2× Master Mix containing DreamTaq^{TM} DNA polymerase (Thermo Fisher Scientific). Prior to visualizing the amplified genes, the PCR products were electrophoresed using agarose gel (1.5%) appropriately stained with ethidium bromide. The position of the gel showing the bands was excised and extracted using Gel DNA Recovery Kit (Zymo Research). Forward and reverse directions sequencing was carried out using BrilliantDye^{TM} Terminator Cycle Sequencing Kit v3.1 (NimaGen, The Netherlands). ABI 3500xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific) was employed to profile the purified fragments. The resulting .ab1 files generated with Genetic Analyzer were further explored using CLC Bio Main Workbench v7.6 through a basic local alignment search tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database [29].

2.8. Partial optimization for enhanced keratinase production

Extracellular keratinase production in MSM was optimized by varying the following variables: initial pH (3–10), incubation temperature (25–30 °C), inoculum size (1–6%, v/v), and feather concentration (5–30 g/L). In each case, the fermentation flasks were incubated with constant agitation (130 rpm) for 72 h. The optimal process variables (temperature 35 °C, pH 5.0, inoculum size 2%, feathers 15 g/L) were used to determine the time course of keratinolytic activity by the bacterial isolate. A portion of the fermentation was taken at intervals of 24 h to determine the kinetics of keratinase titer, bacterial growth, pH change, and protein and sulfhydryl groups' concentrations.

2.9. Determination of the amino acid contents of the chicken feather hydrolysates

Whole chicken feathers were hydrolyzed using keratinolytic isolate in a submerged state fermentation under optimized process conditions. The hydrolysate recovered from the fermentation medium was freeze-dried using vacuum concentrator systems (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and then prepared for amino acid analysis. Digestibility assessment of the feather hydrolysate for amino acids was carried out using 9-fluorenylmethyl chloroformate quantitative derivatization and reversed-phase high-performance liquid chromatography under mild conditions following previously established methods [30,31].

2.10.   Statistical analysis

SigmaPlot version 11.0 was used to analyze the data obtained from the replicate experiments. One-way analysis of variance was used to compare the mean differences at $p < 0.05$ with Duncan's multiple range post-hoc test.

3. Results

3.1. Qualitative evaluation of the bacterial isolates for feather-degrading aptitude

Twelve distinct bacteria were isolated from the soil samples collected from the municipal dumpsite, and six of the isolates showed variable proteolytic activity with halo zone diameters of 35.0 ± 7.07 mm (SSB-03), 30.5 ± 3.53 mm (HSB-02), 29.0 ± 1.41 mm (SSB-02), 17.0 ± 1.41 mm (HSB-04), and 16.0 ± 1.41 mm (SSB-04 and SSB-05). Figure 1a represents skimmed milk hydrolysis by the proteolytic isolate SSB-03. The six proteolytic isolates were further used to initiate chicken feather degradation in MSM (Figure 1b,c). The results show that three of the six isolates (SSB-03, SSB-02, and HSB-02) differentially degraded chicken feathers with a respective percentage feather hydrolysis of 89%, 58%, and 46%.

(A)   (B)   (C)
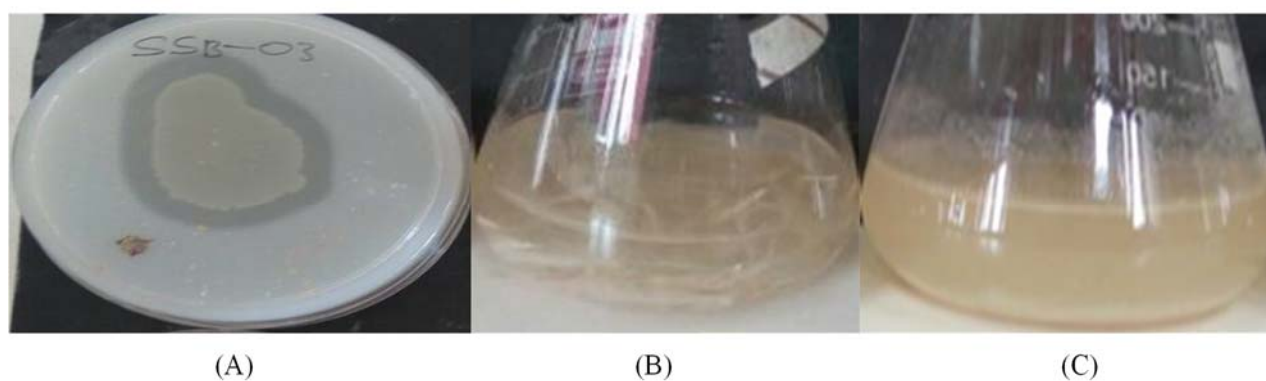
Figure 1. Screening of bacterial isolates for (A) proteolytic activity on skimmed milk. Evaluation of intact chicken feather-degrading capacity of proteolytic bacteria in minimal salt media (B) before fermentation and (C) after fermentation.

3.2. Quantitation of keratinase production and bacterial identification

The extracellular enzyme production by SSB-03, SSB-02 and HSB-02 was evaluated using the cell-free extract of the fermentation (Figure 1c), and the results show respective keratinase activity of 744.5 ± 9.19 U/mL, 269 ± 15.55 U/mL, and 195 ± 7.07 U/mL (Figure 2). Based on the keratinolytic potential of the isolates, SSB-03 was selected for investigations. The 16S rRNA gene sequencing, analysis, and nucleotide blast against the NCBI showed that SSB-03 has 100% and 99.88% sequence similarity with *Exiguobacterium acetylicum* VITNS (NCBI: JX307688) and *Exiguobacterium acetylicum* KIS22 (NCBI: MH017384), respectively. Therefore, SSB-03 was assigned *Exiguobacterium acetylicum* FHBD, and the nucleotide sequence was submitted to GenBank and designated the accession number MW165834.
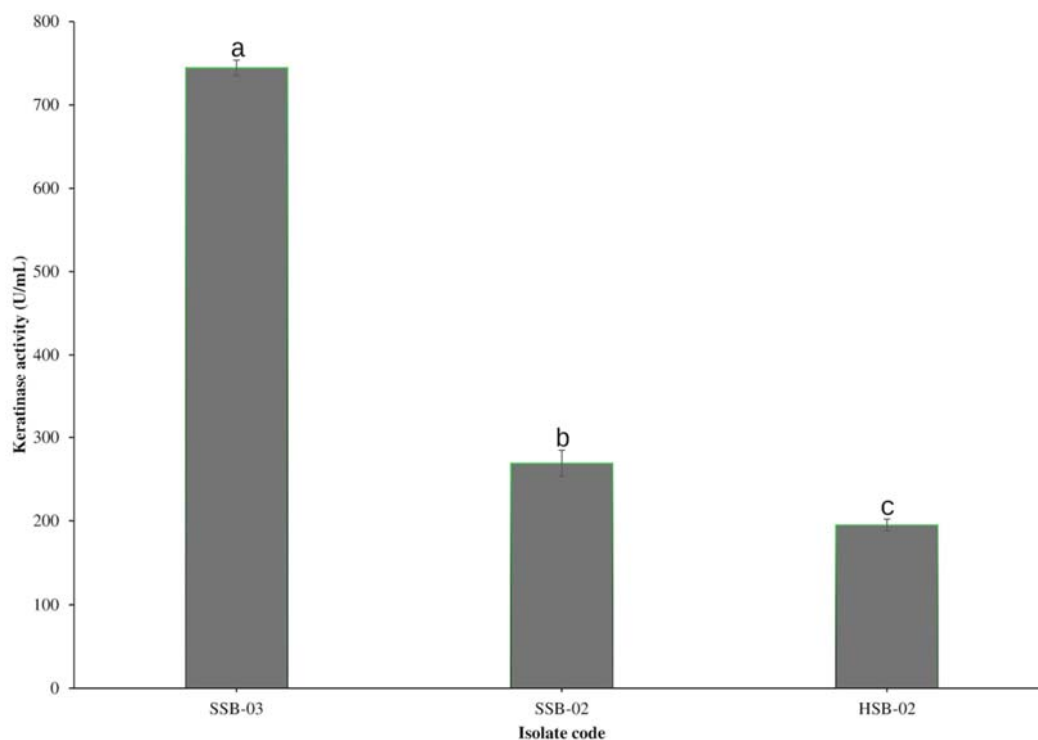
Figure 2. Quantitative evaluation of keratinase activity of bacterial isolates recovered in soil samples from the municipal dumpsite. Each bar denotes the mean plus standard deviation of triplicate experiments. Statistically, bars with similar letter(s) (a,b,c) are not significantly different from each other.

3.3. Optimization of the important variables for enhanced enzyme production

Keratinase production by *E. acetylicum* FHBD was enhanced by varying some physico-nutritional variables. Incubation temperature optimization showed an incremental enzyme production from 333.64 ± 1.29 U/mL and 497.27 ± 1.28 U/mL to 701.82 ± 17.9 U/mL when the temperature was adjusted from 25 and 30 to 35 °C, respectively (Figure 3). However, keratinase production by strain FHBD declined to 297.27 ± 11.57 U/mL when the fermentation was carried out at 40 °C, indicating that 35 °C was the optimal temperature for bacterial keratinase production. *E. acetylicum* FHBD extracellularly secreted keratinase at all pH tested, with optimal enzyme activity of 1492.73 ± 125.99 U/mL at pH 5.0 (Figure 3). Results also show that strain FHBD had more keratinase titer at stronger acidic (pH 3.0) conditions than stronger alkaline conditions (pH 10.0). Changing the size of the starter culture for the fermentation process from 1% to 2% (v/v) resulted in an enzyme production increase from 1415.45 ± 116.99 U/mL to 1699.98 ± 105.42 U/mL. The inoculum was further added to the production medium at concentrations of 3%, 4%, 5%, and 6% (v/v), but there was a corresponding decrease in keratinase production with enzyme activities of 1290.91 ± 41.14 U/mL, 1210.90 ± 71.99 U/mL, 1186.36 ± 96.42 U/mL, and 1029.98 ± 80.95 U/mL compared to the optimum 2% (Figure 3). Fermentation was carried out using different concentrations of chicken feathers to study their effect on extracellular keratinase production by strain FHBD. The results showed that keratinase production increased from 1795.45 ± 47.56 and 1842.73 ± 26.99 to 1997.27 ± 78.43 (U/mL) with increasing

feather concentration from 5 and 10 to 15 g/L, although there was no significant difference (p = 0.471) between enzyme yield at 5 and 10 g/L (Figure 3). Above 15 g/L of chicken feathers, keratinase production decreased drastically with the least enzyme yield (729.09 ± 92.57 U/mL) obtained at 30 g/L (Figure 3).
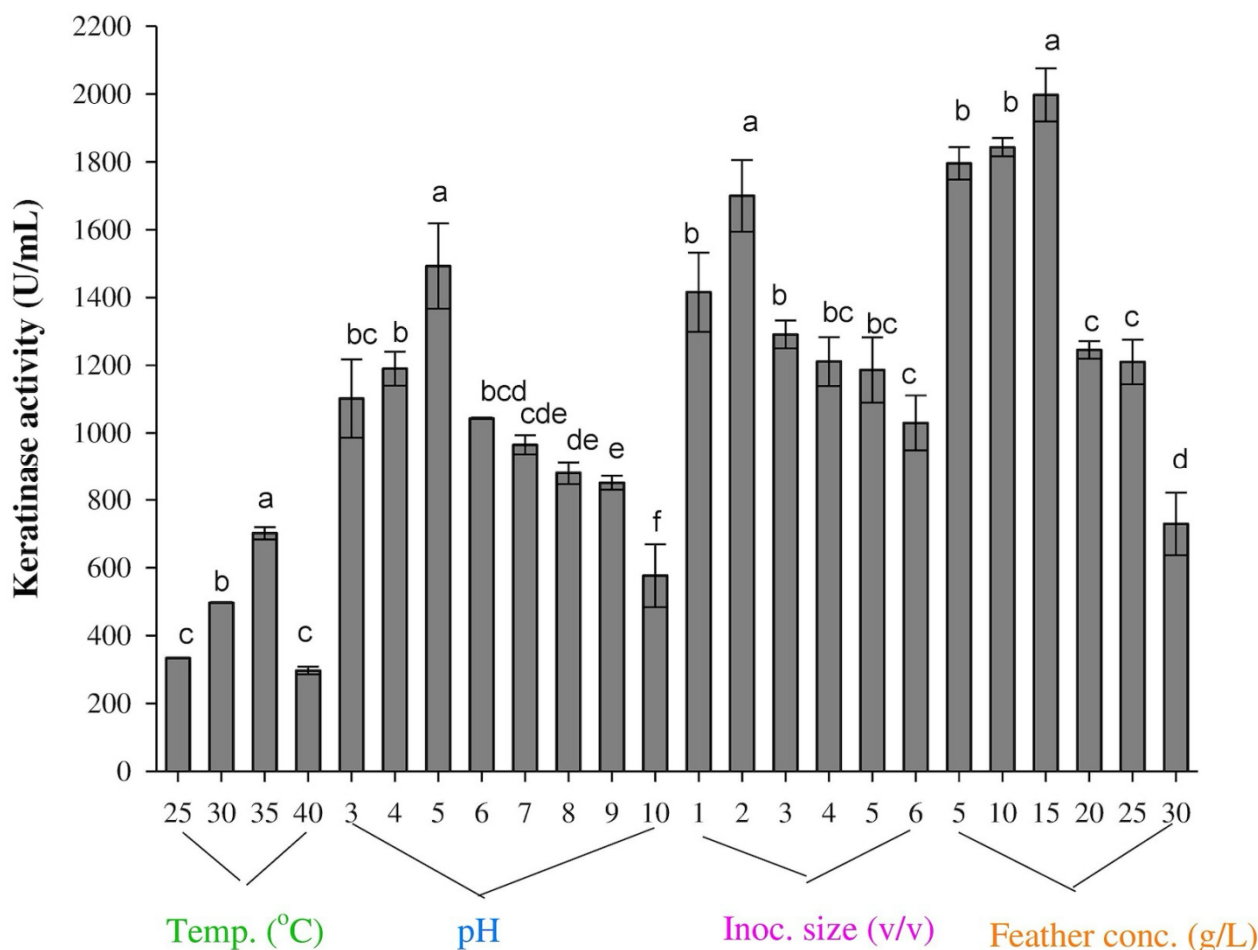

Figure 3. Study of the influence of physico-nutritional variables on extracellular keratinase production by *E. acetylicum* FHBD. Each bar denotes the mean plus standard deviation of triplicate experiments. Statistically, bars with similar letter(s) (a,b,c,d,e,f) are not significantly different from each other.

3.4. Time-dependent study of the keratinolytic activity of the bacterial isolate

The time-course study showed that keratinase production by *E. acetylicum* FHBD increased with an increasing fermentation period, with 608.18 ± 1.29 U/mL, 1180 ± 17.99 U/mL, and 2934.54 ± 38.56 U/mL after 24, 48, and 72 h, respectively (Figure 4). Beyond the optimum production time (72 h), keratinase titer decreased drastically to 1865.45 ± 53.97 U/mL, 896.36 ± 5.14 U/mL, 790.09 ± 2.57 U/mL, and 643.63 ± 38.56 U/mL at 96, 120, 144, and 168 h, respectively. The sulfhydryl groups' content in the fermentation medium showed a similar pattern to the enzyme titer, with a maximum concentration of 3.09 ± 0.02 mM at 72 h (Figure 4). The production medium maintained a constant increment in

soluble protein content with increasing incubation period, recording 1.25 ± 0.05 mg/mL at 168 h (Figure 4). The pH of the fermentation medium was observed to change with increasing incubation time. The initial pH 5.0 was recorded at the start of the fermentation, while 168 h post-incubation the medium pH drifted until with a final value of 8.02 ± 0.01 (Figure 4).
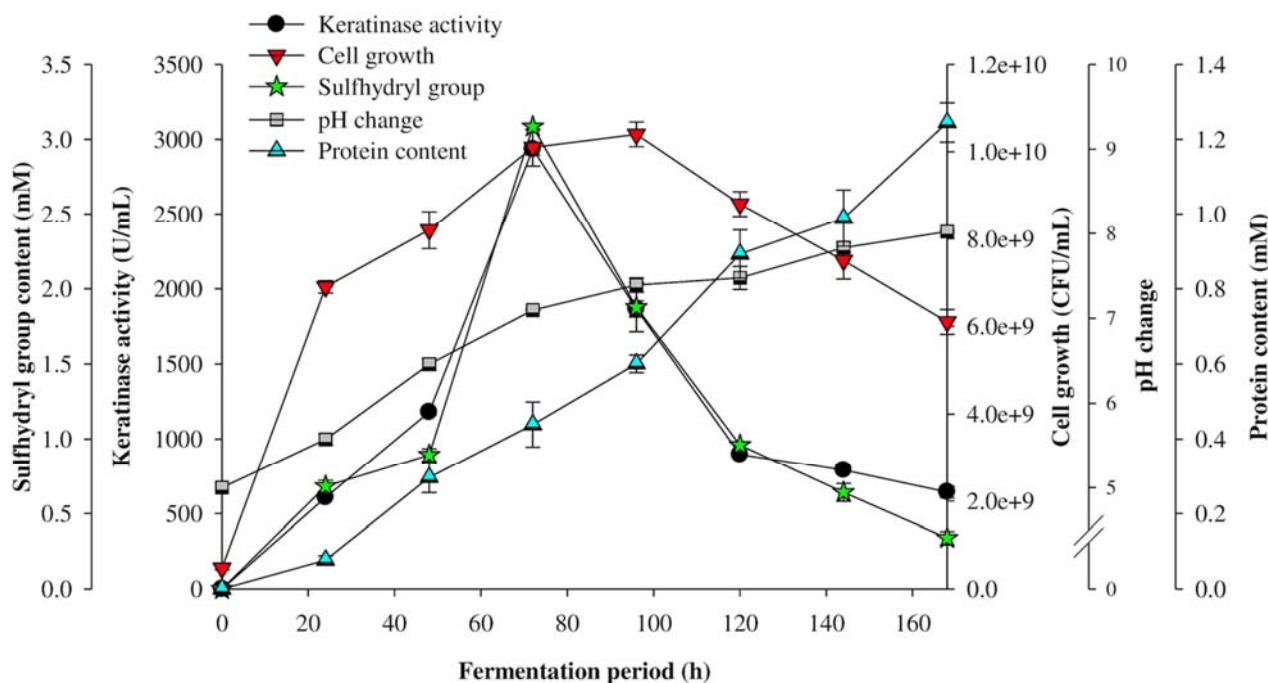
Figure 4. Time-course study of the keratinolytic activity of *E. acetylicum* FHBD at the optimal process conditions. Each point on the line graphs denotes the mean plus standard deviation of triplicate experiments. Error bars are visible when they are bigger than the symbols on the line graphs.

3.5. Amino acid analysis of feather hydrolysate produced by E. acetylicum *FHBD*

The protein hydrolysate generated through the bioconversion of chicken feathers was analyzed for the presence of amino acids. The results indicated that the hydrolysate contained a total protein of 45.04 g/100 g of sample. Among the amino acids detected, glutamic acid, aspartic acid, glycine, arginine, serine, and proline were significantly present with the respective concentrations of 1.58, 1.34, 1.29, 1.20, 1.12, and 0.93 (g/100 g of sample) (Figure 5). In addition, per 100 g of sample, leucine (0.78 g), valine (0.69 g), histidine (0.69 g), lysine (0.68 g), alanine (0.61 g), threonine (0.57 g), and phenylalanine (0.51 g) were also recorded in appreciable concentrations. However, methionine and tryptophan showed the lowest concentrations of 0.05 g/100 g and 0.2 g/100 g, respectively.
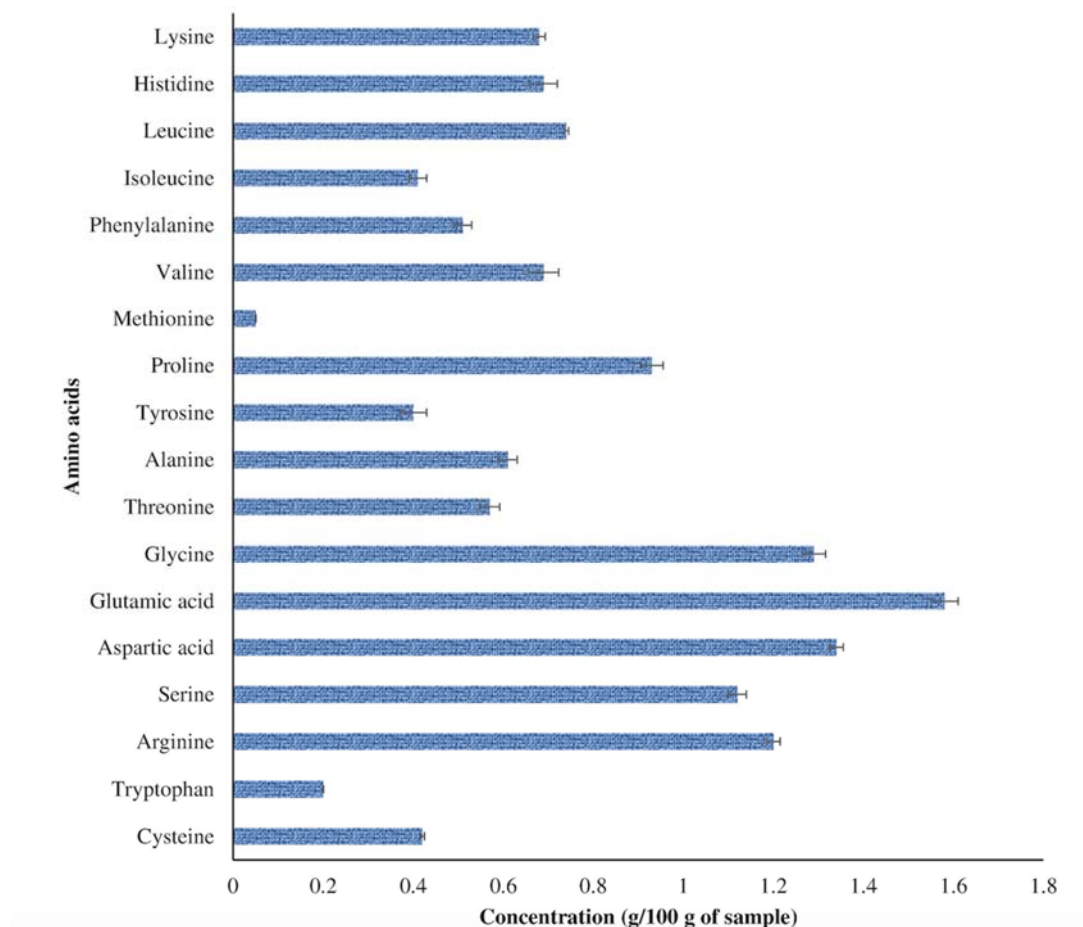
Figure 5. Amino acids profile of protein hydrolysates from chicken feather decomposition by *E. acetylicum* FHBD.

4. Discussion

The ability of microbes to thrive in extreme and constantly changing environments is a testament to their power in creating sustainable ecosystems. A wide range of microbes play a crucial role in implementing clean technology, which promotes a circular bioeconomy. In recent times, poultry feather biodegradation has gained traction due to its numerous benefits in the bioeconomy sector. For this study, feather-degrading bacteria were collected from municipal dumpsite samples, which receive variable waste materials from households and local enterprises. Microbial exposure to different substrates potentially influences the diversity of their metabolic capabilities [32]. The metabolic diversity of the bacterial isolates was evident from their differential proteolytic and keratinolytic activities against the keratinous poultry feather. Among the bacterial strains evaluated, *E. acetylicum* FHBD showed the highest keratinolytic potential, producing extracellular keratinase and degrading feathers effectively. There is limited information on keratinous biomass degradation and keratinase secretion by *Exiguobacterium* spp. [33,34]. Therefore, this study provides valuable insights into the diversity and ecological distribution of keratinolytic bacteria.

At optimized conditions, strain FHBD exhibited thermo-sensitivity within the mesophilic end of the temperature spectrum. The production of enzymes by soil microbes is known to be influenced by

temperature, and the degree of sensitivity can vary among strains [35]. For example, *Bacillus velezensis* ZBE1 showed optimal keratinase production at 35 °C [36], while *Exiguobacterium indicum* secreted maximum keratinase in fermentation medium at 30 °C [34]. The growth of FHBD and its extracellular keratinase production at moderate temperatures highlight its competitive advantage in promoting sustainable development. A previous study found that weakly acidic conditions (pH 5.0) were favorable for keratinase production by *E. acetylicum* FHBD. pH is an important environmental factor that affects microbial growth and metabolism [37], and it also modulates the transport of metabolites from the intracellular compartments to the extracellular milieu by regulating the integrity and transport channels of cellular membranes. The pattern of keratinase production at various pH values tested suggests that strain FHBD's keratinase biosynthetic machinery functions well at acidic pH, as the same microorganism may optimally produce different metabolites at different pH conditions [38]. Other bacteria from environmental samples have shown similar acidic pH optima for exo-keratinase production [39,40].

A suitable starter culture for enzyme production should be in an active growth phase and have a certain size. The viable biomass of strain FHBD showed optimal keratinase production at 2%, indicating that the inoculum concentration was sufficient for rapid cell growth and enzyme production. However, the decremental pattern of keratinase production with increasing inoculum size might be due to the quick depletion of essential nutrients and dissolved oxygen in the production medium caused by an overpopulated culture biomass [41]. A similar inoculum optimum was reported for keratinase production by *B. velezensis* ZBE1 [36] and *Bacillus subtilis* KD-N2 [42]. However, *Bacillus oleronius* [43] and *Arthrobacter* sp. NFH5 [44] produced maximum keratinase at 5% inoculum size, which suggests that strain peculiarity and fermentation medium composition could also influence the required starter culture. The optimization of chicken feather concentration used for keratinase production demonstrated incremental enzyme yield with increasing medium feather concentration until the optimum. This finding highlights the inducibility of the synthesis and secretion of higher keratinase titers for the degradation of increasing concentrations of keratinous biomass for bacterial nutrient acquisition [45]. However, extracellular keratinase production declined above the optimum chicken feather concentration, which might be attributed to the viscous fermentation medium resulting from excessive insoluble substrates that also affect the medium's dissolved oxygen necessary for bacterial growth and respiration [17]. Other bacterial strains reported for their preference for similar or variable concentrations of chicken feathers for maximum keratinase production include *Bacillus tropicus* Gxun-17 (15 g/L) [46], *Thermoactinomyces* sp. YT06 (10 g/L) [47], and *S. maltophilia* DHHJ (25 g/L) [48]. This variation in optimal feather concentrations also alludes to microbial strain differences and genetic diversity.

The highest keratinase activity was observed during the stationary phase of *E. acetylicum* FHBD growth. The molecular changes that occur during this phase, such as increased protein synthesis, are crucial for maintaining the bacterial cells' viability in a nutrient-depleted medium [49]. However, the accumulation of toxic products in the production medium is a common occurrence during the stationary phase [50], which could explain why the enzyme titer declined significantly after 72 h. The optimal timeline for keratinase production may also depend on the bacterial strain and medium composition. Consequently, under submerged state fermentation, *P. geniculata* H10 [14], *Chryseobacterium* sp. RBT [15], *B. velezensis* ZBE1 [36], *B. cereus* HD1 [12], and *S. netropsis* A-ICA [17] achieved the maximum keratinase production of 36.3 ± 2.7 U/mL, 89.12 U/mL, 11.65 U/mL, 41 U/mL, and 113.6 ± 5.1 U/mL, respectively, at different time intervals

of 24, 48, 96, 120, and 120 h, respectively. The highest enzyme activity coincided with the highest medium sulfhydryl group concentration, suggesting that optimal disulfide bond reduction might be facilitated by the increased keratinase titer.

The cleavage of densely populated disulfide bonds in keratinous biomass enhances its biodegradation by exposing the peptide bonds to peptidase attacks [51], making strain FHBD a potential candidate for keratinous biomass valorization. The accumulation of sulfhydryl groups in the fermentation medium during *E. acetylicum* FHBD growth on poultry feathers supports the proposed synergistic mechanism of keratin biodegradation by sulfolytic and proteolytic systems [52]. Efficient dismemberment of keratin-rich biomass into soluble proteins by microbial/enzymatic action causes a medium pH drift toward the alkaline end of the spectrum by causing the deamination of degradation products releasing ammonia in the medium [15]. Similar pH changes have been reported for *Bacillus* sp. CN2 [19] and *Ectobacillus* sp. JY-23 [53] growing on poultry feathers. Additionally, the consistent rise in protein concentration in the fermentation medium following increased incubation time is expected during keratin bioconversion by a competent isolate [14,19].

The amino acid profiling of the feather hydrolysate indicates that *E. acetylicum* FHBD could be a relevant candidate for sustainable development in the agri–food–environmental venues. The total amino acid content was significantly lower than the total protein content of the hydrolysate, and this difference might be attributed to the other peptide constituents that were not completely dismembered into amino acids [5]. The use of cheap protein supplements from agro-residues for livestock feed formulations has been proposed to reduce the cost of poultry production by decreasing food-feed competition and promoting resource use efficiency, thereby bolstering global food security [54]. Different types of bacterial keratinases exhibit distinct patterns of amino acid release from poultry feathers, which depend on their ability to effectively reduce disulfide linkages, recognize peptide bonds, and hydrolyze polypeptide chains [55,56]. Hydrolysates derived from feathers through microbial or keratinase degradation offer significant value in the production of animal feed, as they provide essential amino acids for optimal growth and development of livestock. This assertion was supported by the recent report where feather meals prepared through microbial fermentation offered great nutritional requirements to broilers compared to feather meals prepared by other forms of treatment [57]. Microbial degradation of keratinous feathers requires less energy investment and is environmentally friendly. Contrariwise, feather hydrolysates were prepared using high concentrations of NaOH and HCl [58], and this unsustainable method of managing feather biomass could have adverse consequences on the environment through chemical toxicity.

The byproducts of extraction of keratinases, amino acids, and peptides from feather hydrolysates have shown great relevance, including their use as organic fertilizers in the amendment of soil fertility and as feedstock in biogas production, among others. The waste materials generated from keratinous feather recycling have been reported to possess not only high nitrogen content but also important plant growth hormones, while microbial biomass can aid in nutrient recycling and availability in the soil [59]. In bioenergy production, the microbial-generated feather biomass serves as the pretreated substrate by providing the methanogens with important metabolites and nutrients that enhance their activity during methanogenesis [60]. The metabolic capabilities of *E. acetylicum* FHBD make it a promising candidate for the biodegradation of sturdy feather biomass into a digestible protein source of industrial importance.

5. Conclusions

In conclusion, a bacterial isolate obtained from a municipal dumpsite sample was able to disintegrate keratinous feathers by 89% in a minimal salt medium through biosynthesis and extracellular keratinase production. The isolate was identified as *E. acetylicum* FHBD using a molecular identification system. The isolate demonstrated optimal performance at a temperature of 35 °C, a pH 5, an inoculum size of 2%, and a feather concentration of 15 g/L. The maximum keratinase titer (2934.54 ± 38.56 U/mL) was achieved at 72 h, which corresponded to the time of highest concentration (3.09 ± 0.02 mM) of sulfhydryl groups in the production medium. This suggests that the extensive secretion of keratinolytic enzymes is critical for efficient keratin dismemberment, highlighting the potential of strain FHBD in the circular bioeconomy. The abundance of various amino acids in the feather hydrolysates indicates the relevance of FHBD in sustainable agro-waste valorization and keratinase production. Therefore, further studies on molecular optimization and genetic engineering are imperative to create an industrially competent and robust strain for scale-up applications.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Nnolim Nonso, Uchechukwu Nwodo; Methodology: Tutuka Dlume, Nonso Nnolim; Formal analysis and investigation: Nonso Nnolim; Writing-original draft preparation: Tutuka Dlume; Writing-review and editing: Nonso Nnolim, Uchechukwu Nwodo; Funding acquisition: Uchechukwu Nwodo; Resources: Uchechukwu Nwodo; Supervision: Nonso Nnolim, Uchechukwu Nwodo.

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