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Assessing the Plant Growth-Promoting Potential of Diverse Feather Protein Hydrolysates Produced by Keratinolytic Bacteria

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Poultry slaughterhouses generate substantial quantities of feather waste, posing potential environmental pollution. These feathers can be degraded by microorganisms, yielding amino acid-rich protein hydrolysates. This research aims to assess and compare the microbial degradation rates of feathers from chickens (CK), guinea fowls (GF), and pigeons (PG) for generating amino acid-rich hydrolysates for cowpea growth promotion. Feather-degrading bacteria were isolated from diverse sources, and their capacity to degrade feathers was evaluated by examining keratinase yields, degradation rate, and amino acid composition. Physico-chemical conditions were optimised using a one-factor-at-a-time approach to enhance keratinase yield, while the growth-promoting potential of the different hydrolysates on cowpea was assessed in a pot experiment in a screen house. The results revealed that *Lysinibacillus* sp., from the soil of a slaughterhouse dumpsite, completely degraded 1g/L of GF within 7 days, followed by CK and PG within 8 days. Following optimisation, the keratinase yield improved to 190 U/ml after the degradation of 2g/L of feathers under optimal conditions of pH 8 and temperature of 40°C. The hydrolysate derived from CK exhibited higher amino acid content compared to that from GF and PG, with the exception of alanine, which was more abundant in the GF hydrolysate. All the hydrolysates contained eight essential amino acids, which significantly enhanced various growth parameters of cowpeas, including root length, shoot length, root nodules, leaf area, chlorophyll and leaf numbers after four weeks of planting. The study suggests that *Lysinibacillus* sp. is a promising isolate for efficiently degrading feathers to produce protein hydrolysates, potentially aiding the growth of cowpeas.

Keywords: kearatinase; *Lysinibacillus* sp.; feather degradation; cowpea growth promotion

I. INTRODUCTION

To meet the dietary needs of the ever-expanding global population, there has been a substantial surge in the consumption of various bird meats in recent years. This surge, however, has led to a significant increase in feather waste, primarily generated by chicken slaughterhouses and farms. These feathers constitute a considerable portion of municipal solid waste, which often ends up in open fields and landfills, particularly in many developing nations (Tesfaye *et al.*, 2017). Similarly, feathers discarded from various bird species, such as Guinea fowls, geese, ducks, and a wide variety of birds, pose potential environmental and health concerns. These feathers can serve as hosts for pathogenic microorganisms like *Vibrio* and *Salmonella* and emit gases like ammonia, nitrous oxide, and hydrogen sulphide, thereby negatively impacting both human health and the environment (Grazziotin *et al.*, 2007; Yusuf *et al.*, 2016). Despite these challenges, feather waste has found diverse applications, including heavy metal biosorption (Abba *et al.*, 2020; Yusuf *et al.*, 2020; Al-Asheh *et al.*, 2003), animal feed formulation (Kshetri *et al.*, 2017), electricity generation (Yusuf *et al.*, 2020; Chaturvedi & Verma 2014) and proteinase production (Ferrareze *et al.*, 2016).

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The keratin protein present in feathers exhibits remarkable insolubility in water, weak acids, and organic solvents. Furthermore, it is resistant to degradation by common proteolytic enzymes such as trypsin or pepsin due to the presence of disulfide bonds, hydrogen bonds, salt linkages, and numerous cross-linkages (Kshetri *et al.*, 2017; Yamamura *et al.*, 2002). Additionally, the colouration of feathers can influence their susceptibility to microbial degradation. While some studies indicate that melanised feathers are more challenging for microorganisms to degrade (Gunderson & Frame, 2002; Okoroma *et al.*, 2012), there are exceptions, as Yusuf *et al.* (2020), reported a bacterium with a preference for melanised (black) feathers and also identified bacteria capable of breaking down guinea fowl feathers.

Considering the imperative of feeding the growing African population, enhancing crop yields like cowpea is of paramount importance. Moreover, exploring cost-effective methods of soil fertilisation is becoming increasingly essential. Nitrogen, a vital element for cowpea development, exists in the soil in various chemical forms, including inorganic nitrate (NO_3^-) and ammonium (NH_4^+), or complexed within organic molecules as proteins and amino acids (Jämtgård *et al.*, 2010). Studies have demonstrated the feasibility of plants absorbing nitrogen in organic forms, particularly simple forms like amino acids (Teixeira *et al.*, 2018; Ge *et al.*, 2009). The uptake of amino acids by plants is more efficient, requiring less energy compared to the absorption of NO_3^-, NH_4^+, or nitrogen fixation, which demands energy for converting absorbed nitrogen into amino acids. (Teixeira *et al.*, 2018; Jones & Kielland, 2002) Furthermore, the absorption of amino acids by roots is linked to their availability in the rhizosphere and the activity of amino acid transporters in cell membranes exposed to the soil solution (Jones & Kielland, 2002).

Hence, our hypothesis is that the presence or absence of melanin and other colour agents in various feathers may influence the quantity and composition of amino acids within them, subsequently impacting their potential to promote plant growth. Additionally, the varying mineral content, including Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), along with peptides in feathers, could offer a cost-effective means to enhance cowpea yields. Therefore, this study investigates the ability of locally isolated keratinase-producing bacteria to degrade feathers from guinea fowls, chickens, and pigeons under optimal conditions. Furthermore, we evaluate how the amino acid composition of feather hydrolysates affects the promotion of cowpea growth.

II. MATERIALS AND METHOD

A. Sample Collection and Processing

Feathers from guinea fowl (GF), chicken (CK), and pigeon (PG) were collected from local slaughterhouses in Kano Metropolis, Kano State, Nigeria. Additionally, soil and poultry droppings samples were collected from dumpsites and stored in plastic bags. The feathers underwent a thorough washing with detergent, drying in an oven, and were subsequently cut into pieces before being stored in plastic zip bags at room temperature (Yusuf *et al.*, 2016).

B. Isolation and Selection of Feather-Degrading Bacteria

Soil samples (1 gram each) were suspended in 9 ml of sterile distilled water and serially diluted five-fold using sterilised distilled water. A 50 µl aliquot from dilutions of 10^{-3}, 10^{-4}, and 10^{-5} was spread on freshly prepared skim milk agar (SMA). The SMA medium consisted of g/L 0.5 peptone, 0.3 yeast extract, 0.1 dextrose, 1.0 skim milk powder, and 15 agar in 1L of distilled water, with a pH adjusted to 7.5 (Yusuf *et al.*, 2016). Incubation of plates was carried out at 37 °C for 18 hours. Bacterial colonies displaying substantial hydrolysis zones on the SMA plates were further inoculated onto feather meal agar (FMA), consisting of 1.0 g/L of feather, 0.5 NaCl, 0.7 K_2HPO_4, 1.4 KH_2PO_4, 0.001 $MgSO_4$. $6H_2O$, and 15 agar, to assess their keratin-degrading capability. The confirmation of feather-degrading ability involved growing pure cultures of the isolates in feather meal broth (FMB), as previously outlined by Yusuf *et al.* (2016). FMB composition is the same as FMA except for agar, which is absent in FMB. These cultures were incubated for seven days in a rotary shaker, during which feather degradation rates and keratinase production were analysed. The isolate

demonstrating the highest feather degradation percentage and producing the highest amount of keratinase with at least one of the feathers was selected for further study.

C. Identification of Selected Keratinolytic Bacteria

The selected bacterium was identified using a combination of morphological, biochemical, and molecular techniques. Morphological and biochemical characteristics were confirmed through Gram staining and Bergey's Manual of Systematic Bacteriology, respectively (Cappuccino & Sherman, 1996; Brenner *et al.*, 2005). Molecular identification involving amplification of the 16S rRNA genes was carried out using the 27F (5'-AGAGTTTGATCCTGGCTCAG3') and 1492R (5'-TACGGTTACGTTACGACTT-3') primers, followed by sequencing. The phylogenetic tree was constructed with MEGA 7.0 software using the neighbour-joining method to determine its taxonomy, as previously described (Yusuf *et al.*, 2020; Yusuf & Sharu, 2022).

D. Determination of Feather Degradation Rates

Feather degradation rates were calculated by subtracting the final weight of feathers obtained after incubation from the initial weight placed in FMB (Williams & Davies, 1976). This was achieved by harvesting the remaining feathers from the FMB, washing them, and oven-drying them at 60°C on Whatman No. 1 filter paper until a constant weight was achieved. The final weight was measured, and the weight loss was calculated using the formula:

$$\%\ Feather\ degradation = \frac{X-Y}{Y} x\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(1)$$

where x is the initial weight of feathers, y is the final weight of feathers.

E. Keratinase Assay

The quantification of keratinase produced by bacteria during feather degradation was determined using the method previously described (Yusuf *et al.*, 2016; Yusuf *et al.*, 2019). Briefly, soluble keratin derived from chicken feathers was prepared by heating white chicken feathers in dimethyl sulfoxide, followed by precipitation with cold acetone. The resulting precipitate was dissolved in NaOH, pH adjusted, and diluted to create a buffer solution. For the keratinase activity assay, 1.0 mL of properly diluted crude enzyme was incubated with 1 mL keratin solution at 50°C for 10 min. The reaction was stopped with trichloroacetic acid, and after centrifugation, the absorbance of the supernatant was measured at 280 nm against a control. 1 unit (U/mL) of keratinase activity was defined as a specific increase in corrected absorbance at 280 nm per minute under the specified conditions.

F. Optimisation of pH, Temperature, and Feather Concentration

The impact of initial pH, temperature, and initial feather concentration was investigated by one factor at a time approach. The initial pH of FMB was varied from 6.5 to 11 using a buffer overlapping method, exploring optimum pH for the highest keratinase activity in different buffers, including 50 mM each of acetate buffer (pH 4-6), Tris-HCl (pH 6.5-8), phosphate buffer (pH 7–8), and glycine-NaOH (pH 9–10). Keratinase activity was measured as previously described, and the optimum pH was determined. For temperature optimisation, FMB was adjusted to the optimum pH and incubated at various temperatures (20, 25, 30, 35, 40, 45, 50, and 55°C). Finally, optimising feather concentration involved varying the amount of feathers added to FMB (1-4 g/L) while maintaining the pH and incubation temperature at the previously determined optimum conditions.

G. Amino Acids Determination

The amino acid profiles of the hydrolysates produced from each feather type were determined using an amino acid analyser (Applied Biosystems, US). Norleucine served as an internal standard for all analyses.

H. Greenhouse Cowpea Cultivation

Cultivation was conducted during May–June 2021 in a greenhouse located within the Biological Science Department of Bayero University Kano, Nigeria. Sterilised soil collected from the biological garden of the department was used for cowpea cultivation. The physico-chemical characteristics of

the soil, including clay, silt, sand, organic matter, and mineral contents were determined based on the standard methods. Four surface-sterilised cowpea seeds were planted in pots containing 8 kg of sterilised soil. Cultivation was conducted for 30 days at ambient temperature under natural light, with daily irrigation using sterilised distilled water. Seeds were considered germinated when their radicles extended more than 2 mm.

I. Preparation and Application of Hydrolysates in Plant Growth Promotion Experiment

The hydrolysates produced from each feather type after degradation were centrifuged at 10,000 rpm for 10 min at 4 °C and filtered using a bacteriological-grade sintered filter (0.45 mm, Millipore, USA) to obtain bacteria-free supernatant. Different amounts of hydrolysate (10%, 20%, and 30%) were prepared in sterile distilled water and applied to the seedlings after germination at a rate of 5 ml per 100 g of dry soil. Pots containing soil were mixed with FMB instead of hydrolysates and used as controls in triplicate for each treatment.

J. Measurement of Cowpea Vegetative Growth Parameters

To assess the impact of the treatments, various parameters, including stem circumference, number of leaves, chlorophyll content, number of root nodules, root length, shoot length, and leaf area, were measured 6 days after the initial application of hydrolysates, and subsequently until day 30 (Wang *et al.*, 2016). The circumference of stems was measured using a strip of paper wrapped around the stem, marked where it overlapped, and the length from the mark to the end of the paper was measured using a ruler. The number of leaves was periodically assessed for all cowpeas during cultivation. On day 30, the chlorophyll content of cowpea leaves was measured using a CCM-200 Plus chlorophyll content meter (Apogee Instruments, Inc.). Three leaves per plant were selected, and three measurements were taken. The average chlorophyll content (CCM-200 value) was calculated per treatment. The number of root nodules was counted by uprooting two plants from each treatment, carefully removing soil, and counting the root nodules. The dry weight in grams (DW, g) of cowpea roots, shoot, and leaf length was determined after drying at 65 °C until a constant weight was achieved. The mean dry weight was calculated for each treatment. The leaf area was estimated using a portable leaf area meter. Plant height, root length, and shoot length were measured in centimetres using a meter rule. The number of leaves was determined by counting the number of leaves per plant.

K. Statistical Analysis

Average data and standard deviations were obtained from triplicate experiments for each run using Microsoft Excel (Office, 2019). The standard deviation for each value was ≤5%. Differences in degradation and keratinase production between two or three feather types were analysed using Student's t-test and one-way ANOVA with Minitab version 17. Tukey's post-hoc analysis was employed after ANOVA to identify differences in degradation/keratinase activities between feather groups.

III. RESULTS

A. Screening of Feather-Degrading Bacteria

In our screening process, we successfully isolated a total of 7 feather-degrading bacteria (B, C, D, E, F, G, H) with the capacity to break down at least one of the three feather types. Interestingly, two of these bacterial isolates (C and H) exhibited the ability to degrade all three feather types at higher rates. Detailed information regarding the keratinase activity and feather degradation rates of these isolates can be found in Table 1. Notably, among these isolates, the one designated as Isolate H stood out as it demonstrated a notably faster rate of feather degradation and produced the highest levels of keratinase, ranging from 191 to 196 U/ml. Consequently, Isolate H was selected for further investigation in this study.

Table 1. Screening of feather-degrading bacteria for feather degradation and keratinase production

Isolate code	Feather Types	Feather Degradation (%)	Keratinase activity (U/ml)	Complete degradation (days)
B	Guinea fowl	53±0.0	141± 0.8	7
	Pigeon	36±1.6	145± 0.9	7
	Chicken	51±2.6	155± 0.2	7
C	Guinea fowl	72±3.4	154+0.5	7
	Pigeon	84±0.5	155±0.2	7
	Chicken	63±1.1	157±5.3	7
D	Guinea fowl	62±0.2	156±0.1	4
	Pigeon	73±0.1	155±0.6	4
	Chicken	35±0.0	153±0.0	4
E	Guinea fowl	12±0.0	139±0.7	7
	Pigeon	11±0.0	135±2.5	7
	Chicken	06±0.0	38± 4.3	7
F	Guinea fowl	70±0.2	155±6.2	7
	Pigeon	10±0.1	138±4.2	7
	Chicken	53±0.0	155±2.1	7
G	Guinea fowl	30± 3.2	144±0.5	7
	Pigeon	0±4.1	137±0.5	7
	Chicken	20±3.1	141±0.1	7
H	Guinea fowl	85±2.1	196±0.2	4
	Pigeon	100±5.2	194±4.2	4
	Chicken	90±0.1	191±0.0	4

B. Characterisation, Molecular Identification, and Phylogeny

The selected bacterium exhibited characteristic features, including a Gram-positive nature, a rod-shaped morphology, positive reactions for catalase and oxidase, and negative results for indole and H_2S tests. Through molecular characterisation, the 16S sequence of this bacterium displayed a high degree of similarity, approximately 97%, to that of *Lysinibacillus* sp. A phylogenetic tree depicts the position of the isolate (Figure 1).

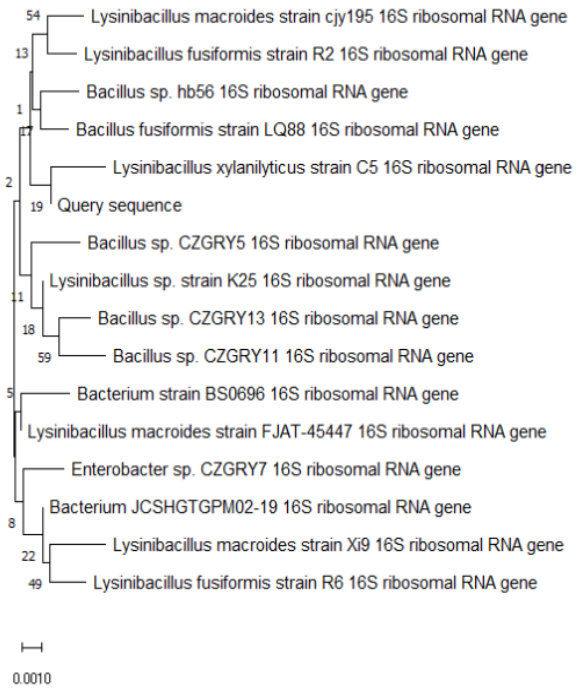

Figure 1. The phylogenetic tree showing relatedness of the *Lysinibacillus* sp. with other closely related species. The percentages of replicate trees where related taxa are clustered together are adjacent to the branches

C. Optimisation of Feather Concentration, pH and Temperature

Our investigation revealed that this bacterium exhibited impressive feather-degradation capabilities. Specifically, when presented with 2 g/L of CK, GF, and PG feathers, it successfully achieved complete degradation in just 4 days, with concomitant production of approximately 196 U/ml of keratinase for each feather type (Figure 2).

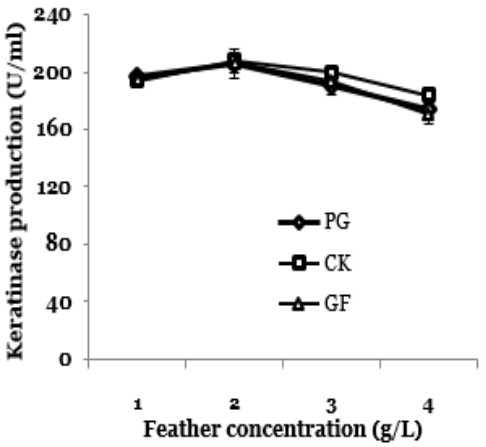

Figure 2. Optimisation of feather concentration in degradation of PG, CK and GF by *Lysinibbacillus* sp.

At pH of 8 (with both Tris-HCl and phosphate buffer), keratinase production exhibited an insignificant increase ($p>0.05$) to approximately 198 U/ml at pH 8 within the same 4-day period. However, deviations from this pH level, either higher or lower, led to reduced rates of keratinase yield (as illustrated in Figure 3).

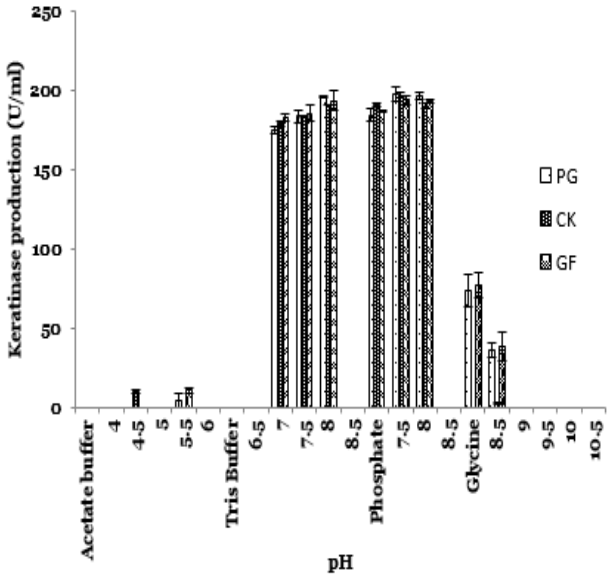

Figure 3. Effect of pH on keratinase production by *Lysinibacillus* sp.

At the optimal temperature of 40 °C, complete degradation was achieved for all feather types within a remarkably shorter time frame of just 3 days, with nearly identical keratinase yields (Figure 3). Remarkably, when we employed a feather concentration of 2g/l complete degradation was once again observed for each feather type, and this was accompanied by a slight increase in keratinase yields, reaching around 200 U/ml for all feather types. However, when feather concentrations exceeded 3 g/l keratinase yields remained consistent, while the rate of feather degradation exhibited a slight reduction to approximately 90% in 3 days, ultimately reaching complete degradation within 4 days.

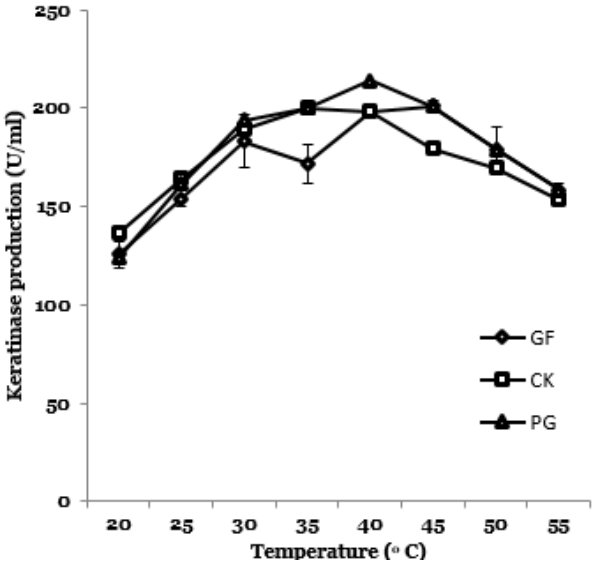

Figure 4. Effect of temperature on Keratinase production by *Lysinibacillus* sp.

D. Amino Acid Contents of CK, GF, and PG Hydrolysates

Table 2 provides insight into the amino acid composition of CK, GF, and PG hydrolysates. To ensure precision, we prepared duplicate samples of hydrolysates from these feathers, and the results indicated excellent agreement between these samples. The mean absolute differences between duplicate samples were minimal. Comparing the amino acid profiles of GF and PG feathers revealed remarkable similarities, while both differed notably from CK feathers. Key distinctions in the amino acid profiles of feather hydrolysates include the following: CK feathers tend to contain approximately twice the concentration of arginine, glycine, glutamic acid, proline, methionine, tryptophan, and lysine when compared to PG and GF feathers. GF feathers exhibit the lowest concentration of methionine, isoleucine, and phenylalanine. Alanine is the least abundant amino acid in CK feathers but is highest in GF feathers. Furthermore, the concentration of all amino acids in CK feathers surpasses that in GF and PG feathers. Essential amino acids, such as phenylalanine, valine, threonine, isoleucine, and leucine, are found in higher amounts in all feathers than histidine, tryptophan, and methionine.

Table 2. Amino acid composition of different feather hydrolysate

Amino acid	CK hydrolysate g/100g protein	GF hydrolysate g/100g protein	PG hydolysate g/100g protein
Leucine	5.15±0.4	4.02±2.0	4.08±0.0
Lysine	4.30±0.0	2.67±1.3	2.62±0.0
Isoleucine	2.62±0.3	1.96±2.0	2.06±1.4
Phenylalanine	3.99±3.4	1.96±2.4	2.30±1.8
Norleucine	INTERNAL STANDARD	INTERNAL STANDARD	INTERNAL STANDARD
Tryptophan	0.6±0.2	0.31±0.0	0.31±2.4
Valine	6.27±0.3	4.70±0.3	4.70±1.1
Methionine	0.86±2.2	0.43±0.4	0.45±0.1
Histidine	0.83±1.9	0.51±3.5	0.45±0.0
Threonine	3.50±2.1	2.99±2.8	2.83±2.1
Tyrosine	1.29±0.0	0.69±2.5	0.69±6.2
Proline	8.54±0.2	3.04±1.0	3.04±1.9
Cystine	5.40±0.3	3.20±0.0	3.14±2.8
Alanine	2.28±0.0	3.60±0.4	3.41±1.1
Glutamic acid	9.55±0.0	5.52±0.3	5.30±2.5
Glycine	8.64±0.3	4.70±2.0	4.69±0.0
Arginine	8.96±2.1	4.13±1.4	4.29±1.0
Serine	5.31±3.2	3.50±1.2	3.55±1.0
Aspartic acid	5.56±1.6	4.15±1.9	4.06±2.9

E. Vegetative Growth Parameters of Greenhouse Cowpea

In this study, we assessed the vegetative growth parameters of greenhouse cowpeas compared to the control group to determine the effects of CK, GF, and PG feather hydrolysates on plant growth. After 30 days, cowpeas treated with various concentrations of hydrolysates displayed increased leaf numbers compared to the control group (see Table 3). This increase was proportional to the concentration of hydrolysates, ranging from 10% to 30%. Remarkably, cowpeas treated with GF hydrolysates exhibited high chlorophyll content, leaf area and leave numbers compared to the other groups.

Table 3. Plant growth activity of cowpeas using different feather hydrolysate treatment

Treatment hydrolysate	Concentration (%)	Circumference of stem (cm)	Number of leaves	Chlorophyll content	Leaf area (cm^2)
Control	0	1.4	7	65	81
CK	10	1.7	15	63.6	133.4
	20	1.7	13	82.6	135.9
	30	1.7	23	87.5	148.8
GF	10	1.7	17	78.5	147.8
	20	1.7	24	73.4	195.2
	30	1.8	26	108.4	129.4
PG	10	1.7	12	79.2	157
	20	1.7	17	99.9	177
	30	1.7	20	103.3	195.3
P-value	*	0.02	0.02	0.14	0.009
	**	0.42	0.05	0.85	0.9

Furthermore, in comparison with the control group, all treatments significantly increased the number of root nodules, root length, and shoot length, as detailed in Table 4.

Table 4. Plant growth activity of cowpeas using different feather hydrolysate treatment

Treatment hydrolysate	Concentration (%)	Root length (cm)	Number of root noodles	Shoot length (cm)
Control	0	15.5	18	15.5
CK	10	20.5	22	21.6
	20	20.5	27	23.5
	30	20.3	33	22.2
GF	10	17	28	23.3
	20	22.2	35	22.3
	30	19.8	39	21.6
PG	10	16.5	28	22.1
	20	20.4	34	21.7
	30	17.3	29	23.3
P-value	*	0.02	0.08	0.00016
	**	0.14	0.49	0.98

*=P-value across the column; **=P-value across the row

IV. DISCUSSION

The escalating production of poultry products has led to a rapid increase in poultry processing industries, consequently resulting in a significant discharge of keratinaceous solid waste in the form of feathers. The indiscriminate disposal of these keratin-containing wastes into the environment has brought about detrimental effects, including nitrate leaching

into groundwater, the release of greenhouse gases into the atmosphere, and the proliferation of bacterial or viral pathogens in waterways (Bhari & Kaur, 2021; Tamreihao *et al.*, 2019). Alternative approaches to waste management involve the conversion of feathers into valuable byproducts such as electricity generation (Yusuf *et al.*, 2020; Chaturvedi & Verma, 2014), heavy metal adsorption (Abba *et al.*, 2019), biogas production (Yusuf *et al.*, 2020; Patinvoh *et al.*, 2016), and their use as biofertilisers (Bhari & Kaur, 2021; Tamreihao *et al.*, 2019). The biodegradation of feathers by keratinolytic microbes is gaining attention among researchers due to its ability to efficiently hydrolyse feathers under mild conditions, yielding hydrolysates rich in peptides and amino acids (Bhari & Kaur, 2021).

In this study, we investigated the degradation of three different types of feathers (chicken, Guinea fowl, and pigeon) using a bacterium isolated from soil, and subsequently utilised the hydrolysates as biofertilisers for cowpea. Various feather-degrading bacteria have been reported in diverse environmental samples, including wastewater, estuaries, poultry processing plants, and effluents (Prakash *et al.*, 2010; Tapia & Simões, 2008). Interestingly, the selected bacterium in this study exhibited the capacity to completely degrade all three feather types, with the highest keratinase production observed with guinea fowl feathers, followed by pigeon and chicken feathers. The differences in degradation rates may be attributed to variations in the composition and structure of the feathers. Chicken feathers, being white, differ from the coloured feathers of guinea fowl and pigeons. Previous studies have reported that bacteria tend to degrade white feathers more rapidly than coloured ones (Gunderson & Frame, 2008; Okoroma *et al.*, 2012). However, other research has identified bacteria that preferentially degrade melanised feathers, such as black feathers from chickens (Yusuf *et al.*, 2020). The ability of our selected bacterium to degrade all three feather types with nearly equal efficiency underscores its potential for keratinous waste management.

Optimising key parameters such as pH, temperature, and feather concentration significantly increased both the initial keratinase yield and the rate of feather degradation. Under optimal conditions (pH 8, 40°C incubation temperature, and 2 g/L of feathers), complete degradation was achieved within 4 days. While many known keratinolytic microbes are mesophiles with feather-degrading activity occurring at temperatures between 25–37 °C, certain studies have reported feather-degrading microbes that are more active at elevated temperatures.

The analysis of amino acids in the hydrolysates revealed that the amino acid profiles in all three feathers were similar, highlighting the genetically conserved nature of feather keratin composition across different bird species. Nevertheless, notable differences were observed between the hydrolysates of chicken feathers and those of guinea fowl and pigeon feathers, primarily due to melanin content. Importantly, all hydrolysates contained essential amino acids, with glutamic acid, glycine, valine, and proline being particularly abundant. The total amino acid content in the hydrolysates was approximately 99.57 wt%, with essential amino acids comprising 42.66 wt%. The study aimed to assess whether amino acid composition varied between feather types and whether these differences could impact the utility of feather hydrolysates as biofertilisers. Our previous study indicated slight differences in most amino acids between intact raw black and white chicken feathers (Yusuf *et al.*, 2020), but the current results revealed significantly lower amino acid concentrations in the hydrolysates. This suggests the loss of some essential amino acids during the degradation process, which could have been utilised by the bacterium. Notably, the amount of glycine in CK hydrolysates increased by 20% compared to intact white feathers (Yusuf *et al.*, 2020), supporting the notion that microbial action can enrich hydrolysates with essential amino acids otherwise absent or present in negligible amounts in intact feathers, such as histidine, methionine, and tryptophan.

Conversely, certain amino acids, such as glycine and cysteine, which were more abundant in raw black chicken feathers, were significantly lower in GF and PG hydrolysates, suggesting potential bacterial assimilation when melanised feathers are used under similar conditions. Cysteine, a readily available essential amino acid in intact melanised and non-melanized feathers, was also notably lower in hydrolysates produced from these feathers. Different feather-degrading bacteria may produce varying concentrations of different amino acids, indicating a role for bacterial specificity. Other

mechanisms, such as direct uptake by bacteria from the environment through ABC transporters/symporters (Guédon & Martin-Verstraete, 2006) or cysteine degradation by L-cysteine desulfhydrase produced by certain bacteria (Yusuf *et al.*, 2020), may also contribute to these variations.

The growth-promoting properties of hydrolysates were assessed on cowpea seedlings, demonstrating that all hydrolysates enhanced cowpea growth compared to the control group. This suggests that the hydrolysates contain amino acids and potentially essential trace elements necessary for plant growth. For example, glutamic acid, found in higher amounts in all hydrolysates, has been reported to stimulate plant growth, activate seed germination, and initiate chlorophyll synthesis. Proline, present at a concentration 100% higher in CK hydrolysates compared to GF and PG hydrolysates, is known to enhance plant immunity under stressful conditions and promote nitrogen accumulation. The most significant cowpea growth-promoting capacity was observed with GF hydrolysates, which contained the highest concentration of alanine. Recent research suggests that β-alanine, an important amino acid, plays crucial roles in nitrogen utilisation efficiency, response to hypoxia, osmoprotection, vitamin B5 and CoA metabolism (Parthasarathy *et al.*, 2019).

Similarly, leucine and valine, present in higher concentrations in all hydrolysates, have been reported to improve plant resistance to high temperatures (Ge *et al.*, 2009). Notably, the growth-promoting ability of hydrolysates was dependent on their concentration. Pots treated with 30% hydrolysates exhibited a 20-50% increase in growth parameters, including the number of leaves, chlorophyll content, leaf area, root length, shoot length, and the number of root nodules, compared to the control group (pots not treated with hydrolysates). The presence of tryptophan and phenylalanine in the hydrolysates may have stimulated auxin synthesis, potentially responsible for the increased root formation in cowpeas. In summary, the presence of hydrolysates rich in amino acids allows cowpea seedlings to access the molecules required for growth without expending additional energy on substance absorption. In addition to amino acids, feathers have been reported to contain usable macronutrients and micronutrients, including phosphorus, calcium, magnesium, iron, zinc, potassium, sulphur, sodium, copper, and manganese, which support plant growth.

V. CONCLUSION

In this study, we successfully degraded feather waste from chickens, guinea fowl, and pigeons using *Lysinibaccilus* sp., which produced substantial amounts of keratinase enzymes. The hydrolysates generated from this process contained both essential and non-essential amino acids, with chicken feathers having higher amino acid content compared to pigeon and guinea fowl feathers. Importantly, the hydrolysates derived from all three feather types significantly enhanced the growth of cowpea seedlings at concentrations of 10%, 20%, and 30%. The bioconversion of various types of feather waste into value-added protein hydrolysates using keratinolytic microbes represents an effective approach for recycling and managing the challenging keratinous waste. Additionally, these hydrolysates serve as a readily available nitrogen source, benefiting the growth of cowpea plants. This research highlights the potential of utilising feather waste as a sustainable resource for both, waste management and agricultural enhancement.

VI. REFERENCES

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