

Research Article

Sustainable Valorization of Poultry Slaughterhouse Waste Using *Bacillus* Strains Isolated from Poultry Manure

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Abstract

Traditional methods for managing poultry slaughterhouse waste, such as landfilling or incineration, are often costly and unsustainable. Therefore, identifying microbial strains capable of producing specific compounds that facilitate waste degradation has become essential. *Bacillus* strains were isolated from poultry manure and selected based on their enzymatic production capacity. Following molecular identification, *Bacillus* sp. B5J22-F and *Bacillus paramycoides* B4J22-F were selected for keratinolytic activity quantification and the evaluation of antibacterial activity under varying fermentation parameters. The studied strains exhibited optimal keratinolytic activity at pH 8 (33.0 U/mL for *B. paramycoides* B4J22-F and 24.9 U/mL for *Bacillus* sp. B5J22-F) and at a temperature of 45 °C (43.6 U/mL for *B. paramycoides* B4J22-F). *Bacillus* sp. B5J22-F also achieved its peak activity at the same temperature (28.8 U/mL), demonstrating their effectiveness at elevated temperatures a critical factor in composting processes. Regarding antimicrobial assays, *B. paramycoides* B4J22-F exhibited the highest inhibition zone against *E. coli* at 30 °C (10.2 mm) and pH 8 (9.1 mm). It also showed notable activity against *S. aureus* at 30 °C (10.6 mm) and against *B. cereus* at pH 5 (12.9 mm), whereas *Bacillus* sp. B5J22-F displayed significant activity against *S. aureus* at pH 10 (14.5 mm). The *Bacillus* strains selected in this study demonstrated strong potential not only in keratin degradation but also in inhibiting the growth of certain pathogenic microorganisms. These findings support their potential use in the valorization of poultry slaughterhouse waste.

Keywords

Bacillus sp., Keratinolytic Activity, Antibacterial Activity, Poultry Slaughterhouse Waste, Poultry Waste Management

1. Introduction

The management of poultry slaughterhouse waste represents a major environmental and health challenge in Côte d'Ivoire, as in most poultry-consuming countries. These

wastes whether liquid (blood, wastewater, etc.) or solid (feathers, intestines, droppings, claws, intestinal contents, etc.) are not only voluminous [1] but may also contain dangerous

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pathogens [2]. Common waste management methods such as landfilling, incineration, or chemical treatment are often inadequate or unsustainable. They may result in groundwater and soil contamination, increased greenhouse gas emissions, and the occupation of large land areas [3, 4]. Moreover, these methods require specialized facilities and complex handling of solid residues, leading to high waste treatment costs [4]. Conversely, traditional composting—a biological degradation process of organic matter [5, 6]—emerges as a viable alternative. This technique is considered eco-friendly, energy-efficient, and produces high-quality protein yields [7], as exemplified by feather composting which yields nitrogen-rich fertilizer suitable for agriculture and animal feed [8, 9]. In the poultry sector, however, composting is mostly applied to feathers, limiting the management of the full range of slaughterhouse waste [10, 11]. Additionally, the degradation of poultry slaughterhouse waste can be slow due to the presence of keratin in feathers. Keratin, the primary component of poultry feathers, is resistant to degradation by conventional proteases [12].

To overcome the limitations of traditional composting, controlled composting using *Bacillus* strains offers a promising solution for the valorization of solid biodegradable slaughterhouse waste [13, 14]. *Bacillus* is a genus of Gram-positive bacteria known for producing keratinases enzymes capable of degrading keratin. Among them, *B. subtilis*, *B. cereus*, and *B. licheniformis* have demonstrated effective feather degradation [15]. These bacteria can not only accelerate the decomposition of organic waste but also inhibit pathogenic microorganisms present in the waste [16], thereby reducing health risks.

This study aims to isolate and select *Bacillus* strains from poultry manure based on their keratin degradation ability and antagonistic activity against pathogenic strains, with the goal of contributing to a safer and more sustainable poultry waste management system.

2. Materials and Methods

2.1. Sampling and Isolation of *Bacillus* Strains

Poultry manure samples were collected from eighteen farms located in the municipalities of Yopougon, Anyama, Port-Bouët, Songon, and Bingerville in Abidjan (Côte d'Ivoire), chosen due to their intense poultry farming activities [17]. Composting was initiated by gathering the collected samples into three piles of 10 kg each, conducted in large plastic containers for 30 days (4 weeks) as described by Biekre et al., [18]. *Bacillus* strains were isolated during the composting process using nutrient agar supplemented with nystatin (50 µg/L). Colonies exhibiting typical morphological and biochemical characteristics of *Bacillus* were isolated and purified through successive subcultures, then preserved in 20% glycerol.

2.2. Detection of Proteolytic, Lipolytic, and Antibacterial Activities

Proteolytic activity was assessed by using nutrient agar enriched with 0.25% glucose and 10% (w/v) skim milk. Positive reactions were indicated by clear halos around inoculated areas. Lipase production was detected on nutrient agar supplemented with 1% Tween 80, where lipolysis appeared as clear zones surrounding the spots [19].

Antibacterial activity was assessed by spotting the isolated strains onto Mueller-Hinton agar previously seeded with reference pathogenic strains: *Salmonella* sp. (ATCC 700623), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 14579), and *Bacillus cereus* (ATCC 25923), after 24 h incubation at 30 °C on nutrient agar. Zones of inhibition were measured after 24 h incubation at 37 °C [20].

2.3. Molecular Identification

Molecular identification of selected strains was carried out by amplification of the 16S rRNA gene using primers F 5'-AGAGTTTGATCCTGGCTCAG-3' and R 5'-GGTTACCTTGTACGACTT-3'. Genomic DNA was extracted and purified using the EZANA® Food DNA kit (OMEGA BIO-TEK, USA). PCR reactions were performed in 50 µL volumes using FirePol Master Mix (Solis Biodyne, Estonia), with 1 µL of each primer (10 µM), 5 µL of template DNA, and nuclease-free water. Cycling conditions were: initial denaturation at 94 °C for 5 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 70 °C for 1 min, with a final elongation at 72 °C for 10 min. PCR products were visualized by electrophoresis on 1.5% agarose gel in 1X TAE buffer with SYBR® Safe stain (Invitrogen™, USA), using a GelDoc EZ Imager (BIO-RAD, USA). Amplicons were purified with the Invitrogen™ Clean-Up kit before sequencing. Sequences were compared and aligned via BLAST against the GenBank database (NCBI).

2.4. Keratinase Screening and Keratinolytic Activity Assay

To assess feather-degrading ability, a selective medium containing 0.7 g/L KH₂PO₄, 1.4 g/L K₂HPO₄, 0.5 g/L NaCl, 0.06 g/L CaCl₂, and 0.1 g/L MgCl₂ 6H₂O was prepared. One percent (1%) of feathers were added to test tubes containing 5 mL of medium, then sterilized by autoclaving. After cooling, 2-3 colonies from nutrient agar were inoculated and incubated at 30 °C for 7 to 10 days. Feather degradation was assessed visually.

Keratinolytic activity was quantified using keratin extracted from feathers via DMSO, followed by acetone precipitation and distilled water washing. For quantification, 2-3 colonies were inoculated into 5 mL of the previous medium supplemented with feather meal and incubated at 30 °C under

agitation for 72 h. The supernatant obtained after centrifugation at $6000 \times g$ for 20 min at 4 °C was used as the crude enzyme.

The assay consisted of incubating 1 mL of supernatant with 1 mL of keratin solution at 50 °C for 10 min in a water bath. The reaction was stopped by adding 2 mL of 0.4 M trichloroacetic acid (TCA). After centrifugation at 1450 rpm for 30 min, the absorbance of the supernatant was measured at 280 nm. One unit (U/mL) of keratinolytic activity was defined as an increase in OD280 of 0.01 per minute under the assay conditions [21].

2.5. Effect of Fermentation Parameters on Technological Traits

The effects of fermentation parameters, such as temperature, pH, and substrate concentration, on keratinolytic activity and antibacterial potential of the selected strains were evaluated.

2.6. Statistical Analysis

All experiments were conducted in triplicate and results were expressed as mean $\pm$ standard deviation. ANOVA was performed using XLSTAT 2019. Duncan's multiple range test was used to assess significant differences at a 95% confidence level.

3. Results

3.1. Detection of Proteolytic, Lipolytic, and Keratinolytic Activities

A total of 18 strains exhibiting morphological and biochemical characteristics consistent with *Bacillus* spp., were isolated from poultry manure samples.

Among these 18 isolates, two strains (B4J22 and B5J22) were selected based on their significant proteolytic activity (25.33 ± 2.5 mm and 18.66 ± 3.5 mm, respectively), lipolytic

activity (13.66 ± 1.1 mm and 6.83 ± 1.4 mm, respectively), and their ability to degrade feathers (Table 1) (Figure 1).

Table 1. Detection of keratinase, protease, and lipase production.

Strain	Keratinolytic Activity	Proteolytic Activity (mm)	Lipolytic Activity (mm)
Bacillus B4J22	+++	25.33 ± 2.5^a	13.66 ± 1.1^a
Bacillus B5J22	+++	18.66 ± 3.5^a	6.83 ± 1.4^b

Note: Means followed by the same letter in the same column are not significantly different at $p < 0.05$.



Figure 1. Screening for keratinolytic activity. Result obtained after 7 days of incubation with strain B4J22.

3.2. Molecular Identification

Sequencing of the 16S rRNA gene of the isolates confirmed that both selected strains belong to the *Bacillus* genus and were identified as *Bacillus* sp. B5J22-F and *Bacillus paramycoides* B4J22-F ((Figure 2).

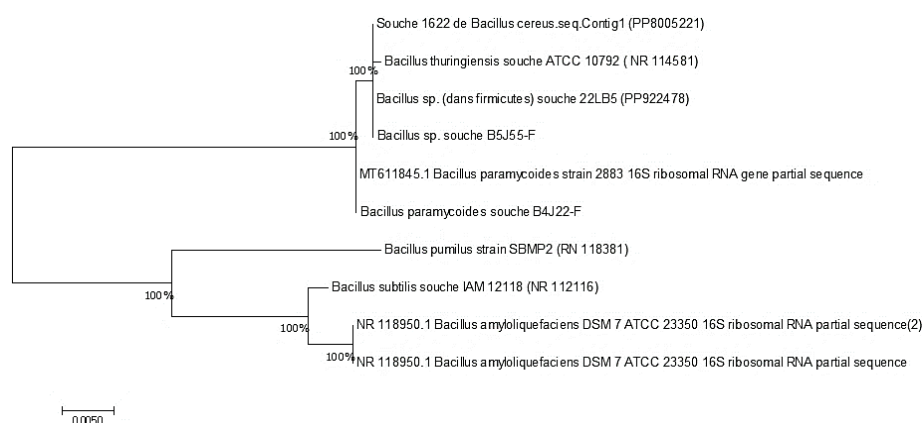


Figure 2. Phylogenetic tree showing the relationships between the selected strains (*Bacillus paramycoides* B4J22-F and *Bacillus* sp. B5J22-F) and other reference strains of *Bacillus* species.

3.3. Keratinolytic Activity and Detection of Antibacterial Properties

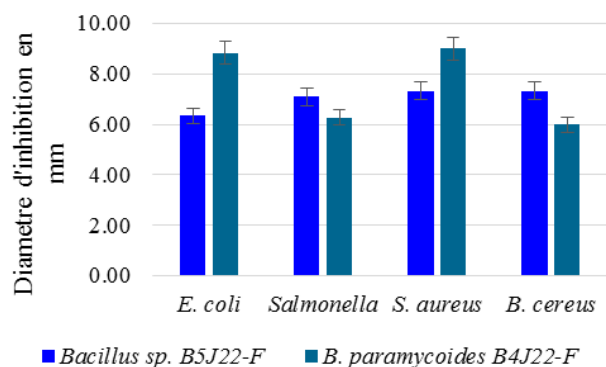


Figure 3. Detection of antibacterial activity.

Antagonistic assays demonstrated that the *Bacillus* strains exhibited antimicrobial activity against all tested reference pathogens. The highest inhibition diameter was recorded for *B. paramycoides* B4J22-F against *S. aureus* (9 mm), whereas *Bacillus sp.* B5J22-F showed an inhibition zone of 8.83 mm against *E. coli* (Figure 3). The keratinolytic activity was 23.40 U/ml for B5J22 and 25.45 U/ml for B4J22.

3.4. Evaluation of Fermentation Parameters on Keratinolytic Activity

The results showed that the selected strains exhibited maximal keratinolytic activity at 45 °C, with 43.6 U/mL for *B. paramycoides* B4J22-F and 28.8 U/mL for *Bacillus sp.* B5J22-F. Optimal activity was also observed at pH 8, with 33.0 U/mL and 24.9 U/mL, respectively (Figure 4).

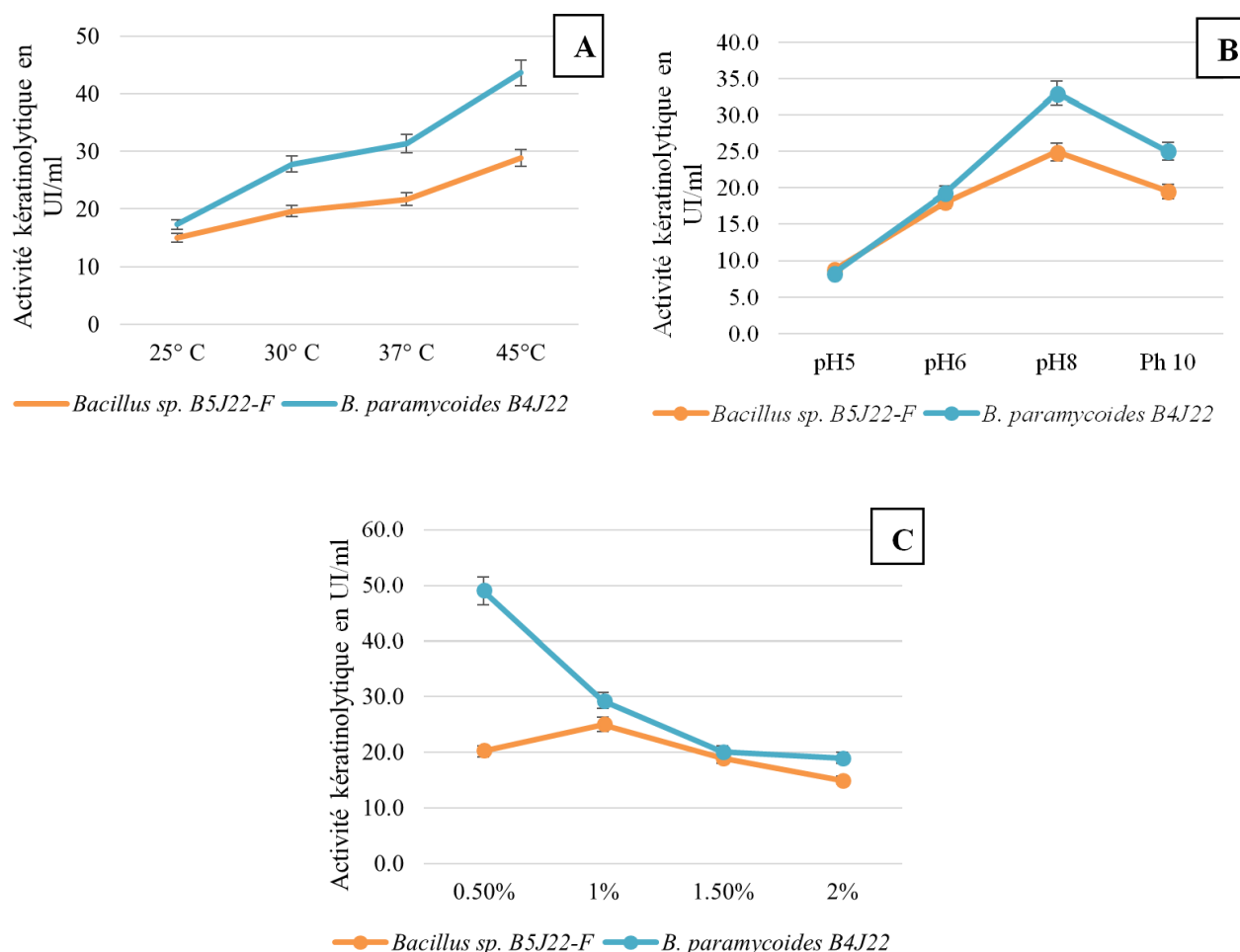


Figure 4. A) Effect of temperature (°C) on keratinolytic activity (U/mL). B) Effect of pH on keratinolytic activity (U/mL). C) Effect of substrate (feather) variation on keratinolytic activity (U/mL).

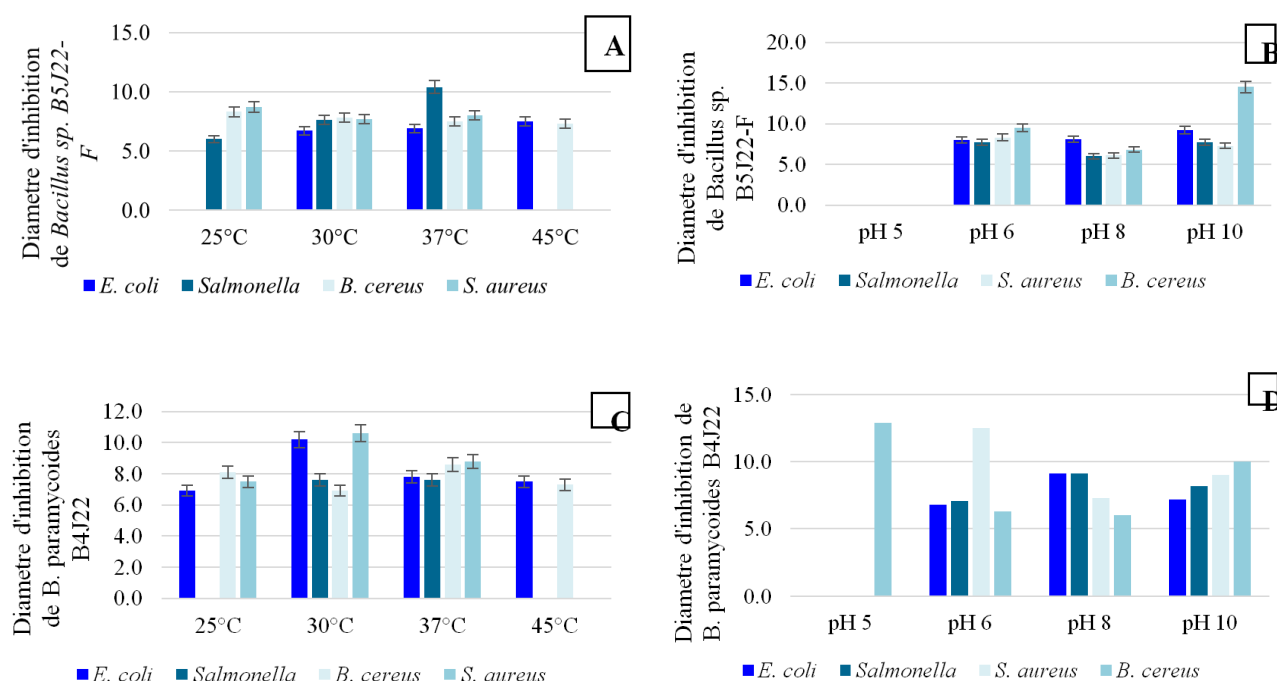


Figure 5. A- B) Effect of temperature (°C) variation and pH on inhibition diameter of *Bacillus sp. B5J22-F* against pathogenic strains. C-D) Effect of temperature (°C) variation and pH variation on inhibition diameter of *B. paramycoides B4J22-F* against pathogenic strains.

3.5. Evaluation of Fermentation Parameters on Antibacterial Activity

B. paramycoides B4J22-F exhibited maximum antimicrobial activity against *E. coli* at 30 °C (10.2 mm) and at pH 8 (9.1 mm). *Bacillus sp. B5J22-F* also demonstrated effective activity against *E. coli* at 30 °C (6.7 mm), with a maximum inhibition at pH 10 (9.2 mm). Moreover, *B. paramycoides B4J22-F* showed an inhibition diameter of 10.6 mm against *S. aureus* at 30 °C and 12.9 mm against *B. cereus* at pH 5. Against *S. aureus*, *Bacillus sp. B5J22-F* exhibited strong activity at pH 10 (14.5 mm) (Figure 5).

4. Discussion

Poultry slaughterhouse waste represents a significant portion of the solid waste generated globally [1]. Consequently, it must be properly treated to exploit its recovery potential and minimize its environmental impact. Current research focuses on developing effective techniques to sustainably manage this waste and ensure its conversion into value-added products. The use of specific microbial strains, such as *Bacillus* spp., capable of optimizing the degradation process, has been proposed as a promising method for poultry waste management [22, 23]. This growing interest in these microorganisms has led to several studies on their isolation and characterization, with the main challenge being the identification of strains exhibiting strong biodegradation capabilities.

In total, 18 strains were isolated from collected poultry

manure samples. Among them, *Bacillus sp. B5J22-F* and *Bacillus paramycoides B4J22-F* stood out due to their ability to produce proteases and lipases, degrade feathers within seven days, and inhibit the growth of reference pathogens.

This performance is unsurprising, as the *Bacillus* genus is widely recognized as the primary group of bacteria involved in keratinous compound degradation. Its ease of handling and potential for large-scale applications have made it a key reference for keratinase production [24, 25]. For instance, *B. licheniformis* is the first commercially exploited species for keratinase production [26]. *B. paramycoides*, on the other hand, is an emerging candidate for feather degradation [25]. It has also been reported for its roles in uric acid biodegradation [27], hospital wastewater bioremediation [28], and antibacterial agent biosynthesis [29].

In this study, the enzymatic efficiency of the selected strains was particularly notable under specific temperature and pH conditions. Regarding antimicrobial activity, both strains effectively inhibited the growth of Gram-negative bacteria such as *E. coli* and *Salmonella*, as well as Gram-positive species like *S. aureus* and *B. cereus*, albeit with reduced performance at high temperatures (45 °C) and under acidic conditions (pH 5) (Figure 5). While the antibacterial activity spectrum of *B. subtilis* TS 01 and *B. subtilis* ZR 02—both isolated from soil—exceeds the present results (up to 50.0 mm) [30], the selected strains clearly demonstrated their ability to inhibit the growth of pathogenic strains. This antagonistic activity is generally attributed to the synthesis of various inhibitory substances such as hydrogen peroxide, bacitracin, bacteriocins, siderophores, and [31, 32].

The two strains exhibited maximum keratinolytic activity at

45 °C (43.6 U/mL for *Bacillus* sp. B5J22-F and 28.8 U/mL for *B. paramycoides* B4J22-F), similar to *B. licheniformis* dcs1 [33]. This highlights the strains' ability to tolerate high temperatures and maintain function under such conditions. Thermotolerance is a crucial trait for poultry waste fermentation. According to Chen et al. [34], temperature is a key indicator of compost maturity—it directly reflects the composting process and microbial activity. At the start of composting, temperature rapidly rises, reaching thermophilic levels (>45 °C), which is essential for the rapid breakdown of organic matter by the compost's microbial community [30].

During this initial composting phase, the pH is generally alkaline (around 8 to 9), due to protein decomposition and ammonia release [30]. As composting progresses, the pH decreases with the formation of organic acids, eventually stabilizing at a more neutral or slightly acidic level—conditions that are less favorable for the strains identified in this study. Indeed, these strains showed maximal keratinolytic activity at pH 8 (Figure 4), suggesting a preference for neutral to mildly alkaline environments for optimal enzymatic activity. This finding is supported by other researchers who have also identified neutral to alkaline pH as optimal for keratinase production by *Bacillus* strains. For instance, *Bacillus* sp. NKSP-7 demonstrated peak activity at pH 7 [35], while *B. cereus* KK69 exhibited optimal performance at pH 9 [10].

Given their ability to degrade keratin, the main component of poultry slaughterhouse waste, the strains investigated here represent promising candidates for the fermentation of poultry waste. This valorization process could enable the production of biofertilizers with minimized pathogenic load, thereby contributing to the reduction of environmental and public health risks associated with this type of waste.

5. Conclusions

This study demonstrates the potential of *Bacillus* sp. B5J22-F and *Bacillus paramycoides* B4J22-F for the sustainable management of poultry slaughterhouse waste. Integrating these bacteria into waste treatment systems could transform an environmental burden into a valuable resource, while enhancing public health and ecological sustainability. However, it is evident that their effectiveness may be influenced by variations in temperature and pH. Therefore, combining them with strains that can tolerate acidic pH or produce potent antibacterial activity may further optimize the biodegradation process of poultry slaughterhouse waste.

Abbreviations

KH ₂ PO ₄	Dipotassium Phosphate
CaCl ₂	Calcium Chloride
MgCl ₂	Magnesium Chloride
E.	<i>Escherichia</i>

S.	<i>Staphylococcus</i>
B.	<i>Bacillus</i>
ATCC	American Type Culture Collection
DNA	Deoxyribonucleic Acid
Rna	Ribosomal Ribonucleic Acid
TAE	Tris-acetate-EDTA
PCR	Polymerase Chain Reaction
DMSO	Dimethyl Sulfoxide

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Author Contributions

Goualie Bernadette: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing

Solange Kakou-Ngazona: Supervision

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Conflicts of Interest

The authors declare no conflicts of interest.

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