

Keratinase Production from Cattle Horn Waste by Abattoir-Derived Bacterial Isolates in Kwara State, Nigeria

¹Majekodunmi Racheal Adedayo, ^{*1}Kate Rachael Imeje, ¹Adeyinka Elizabeth Ajiboye, and ²Frank Olakunle Otuyelu

¹Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Kwara State, Nigeria.

²University of Ilorin, Department of Microbiology, Faculty of Pure and Applied Sciences, Ilorin, Kwara State, Nigeria.

*Corresponding authors' email: kateimeje22@gmail.com

ABSTRACT

Cattle horn is a keratin-rich animal by-product whose compact protein structure makes it resistant to ordinary proteolytic degradation. Microbial keratinases provide a biological approach for converting keratinous waste into soluble products. This study aimed to isolate bacteria from abattoir soil, screen the isolates for extracellular proteolytic activity, identify the selected isolates, and evaluate keratinase activity during submerged fermentation with cattle horn powder. Five soil samples were collected from five spots across two locations at Akerebiata Abattoir, Ilorin, Kwara State, Nigeria, at a depth of 5 cm below the top soil. Six bacterial isolates were recovered on horn meal agar and screened on skim milk agar for extracellular proteolytic activity. The selected isolates were identified by 16S rRNA gene sequencing and BLAST analysis. Keratinase production was evaluated by submerged fermentation in 250 mL vessels containing 100 mL production medium, using 5 mL inoculum at 37 °C and 120 rpm for 14 days. Fermentation was conducted in three biological replicates, and enzyme assays were performed in duplicates. Six bacterial isolates were recovered. A₁ and B₃ produced hydrolysis halos of 18.00 ± 0.04 mm and 22.00 ± 0.025 mm, respectively. BLAST analysis identified isolate K1 as *Bacillus cereus* and isolate K2 as *Lysinibacillus fusiformis*. K1 had a sequence length of 1518 bp, 99.34% identity, 100% query coverage and an E-value of 0.0 against accession GQ406846.1. K2 had a sequence length of 1516 bp, 99.93% identity, 100% query coverage and an E-value of 0.0 against accession OQ568519.1. K1 reached a maximum keratinase activity of 42.88 ± 1.71 U/mL on day 7, whereas K2 reached 28.64 ± 1.12 U/mL on day 5. Cattle horn powder supported measurable keratinase activity by the two selected abattoir-derived isolates under the tested fermentation conditions. The findings provide experimental evidence for the use of cattle horn as a substrate for microbial keratinase production.

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INTRODUCTION

Abattoirs and meat-processing facilities generate keratin-containing by-products such as horns, hooves, feathers and hair, and the extensive disulfide bonding, hydrogen bonding and hydrophobic interactions in keratin contribute to its resistance to ordinary proteolytic degradation (Sypka et al., 2021; Banasaz and Ferraro, 2024).

Cattle horn is a hard mammalian keratinous material, whereas poultry feathers are the substrate used in a large proportion of published microbial keratinase studies (Banasaz and Ferraro, 2024; Das et al., 2024). The present study therefore evaluated cattle horn powder directly as the principal keratin substrate rather than extrapolating findings obtained with feather waste.

Keratinases are extracellular proteolytic enzymes produced by bacteria, fungi and actinomycetes that hydrolyse recalcitrant keratin into smaller soluble products (de Menezes et al., 2021). Their reported applications include keratin-waste treatment, leather processing, detergents and production of keratin hydrolysates (Sypka et al., 2021; Mpaka et al., 2025). Bacterial strains are commonly investigated in submerged fermentation because many keratinolytic bacteria secrete extracellular enzymes into the culture medium (Aktayeva et al., 2022; Rahimnahl et al., 2023).

Abattoir soil was selected because keratin-rich and protein-rich waste environments have yielded bacteria with

extracellular proteolytic and keratinolytic activity, including members of *Bacillus* and related genera (Deba et al., 2023; Gerlicz et al., 2024; Vema and Kumar, 2023). Studies using poultry feathers are more numerous than studies in which cattle horn is used directly as the principal fermentation substrate (Das et al., 2024; Banasaz and Ferraro, 2024).

The study objectives were to: (i) recover bacterial isolates from abattoir soil and screen them for extracellular proteolytic activity; (ii) identify the selected isolates using 16S rRNA gene sequencing and BLAST analysis; and (iii) determine the 14-day time course of keratinase activity during submerged fermentation with cattle horn powder.

MATERIALS AND METHODS

Study Area and Sample Collection

Five soil samples were collected aseptically from five spots distributed across two locations within Akerebiata Abattoir, Ilorin, Kwara State, Nigeria. Samples were collected at a depth of 5 cm below the topsoil, placed in sterile containers and transported promptly to the laboratory for microbiological analysis.

Preparation of Horn Meal Agar

Horn meal agar was prepared per litre using 10 g of finely powdered cattle horn meal, 0.7 g KH₂PO₄, 0.3 g K₂HPO₄, 0.5 g NaCl, 0.1 g MgSO₄ and 15 g agar. The components were

dissolved in 1 L of distilled water, the pH was adjusted to 7.0, and the medium was sterilized before cooling and use.

Isolation and Screening of Keratinase-Producing Bacteria

One gram of each soil sample was transferred into 9 mL of sterile distilled water and mixed to obtain a stock suspension. Each suspension was serially diluted to 10^{-5} by successive 1 mL transfers into 9 mL of sterile distilled water. One millilitre from the 10^{-2} dilution was transferred aseptically into sterile Petri dishes, cooled molten horn meal agar was added, and the plates were incubated at 37 °C for 72 h. Six bacterial isolates were recovered and sub-cultured on nutrient agar to obtain pure cultures (de Menezes *et al.*, 2021).

All six purified isolates were screened on skim milk agar for extracellular proteolytic activity. Casein hydrolysis was recorded as a visible clearing halo around bacterial growth, and the total diameter of the visible clearing zone was measured in millimetres. A₁ and B₃ were selected because they were the only isolates that produced measurable halo zones. Skim milk agar was used as a preliminary proteolytic screen, while keratinase production was subsequently evaluated with cattle horn powder as the keratin substrate (Deba *et al.*, 2023).

Molecular Identification of Selected Isolates

The selected isolates were identified by amplification and sequencing of the nearly complete 16S rRNA gene using the workflow described by Martínez *et al.* (2023). Genomic DNA was extracted from pure cultures, and the 16S rRNA gene was amplified with universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR comprised an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1.5 min, with a final extension at 72 °C for 10 min. Amplicons were subjected to Sanger sequencing through Macrogen, Seoul, South Korea.

The resulting sequences were compared with reference sequences in the National Center for Biotechnology Information nucleotide database using the Basic Local Alignment Search Tool. Sequence length, percentage identity, E-value, query coverage and the accession number of the closest BLAST reference match were recorded for each isolate.

Phylogenetic Analysis

The 16S rRNA gene sequences of the selected isolates and related reference sequences obtained from the National Center for Biotechnology Information database were aligned using CLUSTAL X version 2.0. Phylogenetic relationships were inferred using a maximum-likelihood approach in MEGA version 6.0, following the 16S rRNA analysis workflow described by Martínez *et al.* (2023).

Morphological and Biochemical Characterisation

The selected isolates were examined for colony colour, Gram-stain reaction, cell shape, motility and spore formation. Biochemical characterisation included catalase, oxidase, urease, citrate utilisation, indole, methyl red, Voges-Proskauer, starch hydrolysis, casein hydrolysis, gelatin liquefaction and carbohydrate-fermentation tests (Aktayeva *et al.*, 2022; Vema and Kumar, 2023).

Catalase activity was assessed by observing effervescence after exposure of a fresh culture to hydrogen peroxide. Oxidase activity was assessed with oxidase reagent. Urease activity was evaluated in urea-containing medium, while citrate utilisation was assessed on citrate medium. Indole,

methyl red and Voges-Proskauer reactions were determined with the corresponding reagents. Starch and casein hydrolysis were recorded from clearing around growth, while persistent liquefaction after incubation was recorded as a positive gelatinase reaction. Glucose, lactose and sucrose were used for carbohydrate-fermentation tests, with acid production and gas recorded where present.

Keratinase Production

The production medium contained KH₂PO₄, K₂HPO₄, MgSO₄·7H₂O and NaCl in distilled water, with 1 g of cattle horn powder added per 100 mL as the keratin substrate. The initial pH was adjusted to 8.0–8.2 before sterilization. One hundred millilitres of the production medium was dispensed into 250 mL fermentation vessels and autoclaved at 121 °C for 15 min.

A 5 mL inoculum of each selected pure culture was aseptically introduced into each fermentation vessel, while an uninoculated vessel served as the control. Fermentation was conducted in three biological replicates at 37 °C and 120 rpm for 14 days. Five-millilitre samples were withdrawn repeatedly from the same vessels on days 1, 3, 5, 7, 9, 11 and 14. At each sampling interval, the broth was centrifuged at 10,000 rpm for 15 min, and the cell-free supernatant was collected as the crude enzyme preparation.

Keratinase Enzyme Assay

Keratinase activity was determined using cattle horn powder as the keratin substrate. A 0.5 g portion of cattle horn powder was mixed with 1 mL of crude enzyme preparation and 1 mL of phosphate buffer and incubated at 50 °C for 10 min. The reaction was stopped with 2 mL of trichloroacetic acid, the mixture was centrifuged at 10,000 rpm for 10 min, and absorbance of the supernatant was measured at 280 nm using a UV-visible spectrophotometer (de Menezes *et al.*, 2021; Derhab *et al.*, 2024).

One unit of keratinase activity was defined as the amount of enzyme that produced an increase of 0.01 absorbance unit under the assay conditions. Keratinase activity was calculated as:

$$\text{Keratinase activity (U/mL)} = (4 \times n \times \Delta A_{280}) / (0.01 \times t)$$

where n is the dilution factor, ΔA_{280} is the change in absorbance at 280 nm and t is the incubation time in minutes.

Data Analysis

Fermentation was conducted in three biological replicates and analytical keratinase activity measurements from each withdrawn sample were performed in duplicate. Quantitative results were expressed as mean $\pm$ standard deviation (SD). Descriptive statistics were generated using IBM SPSS Statistics. No inferential statistical test was applied to the time-course data.

Biosafety and Ethical Statement

The study did not involve human participants or live experimental animals. All culture work was confined to laboratory-scale microbiological procedures, and the bacterial isolates were not used for food, feed, field or environmental application

RESULTS AND DISCUSSION

Isolation and Proteolytic Screening of Bacterial Isolates

Six bacterial isolates were recovered on horn meal agar and screened on skim milk agar. Only A₁ and B₃ produced measurable clearing zones. The mean total clearing-zone diameter was 18.00 ± 0.04 mm for A₁ and 22.00 ± 0.025 mm

for B₃. These two isolates were selected for subsequent keratinase production with cattle horn powder.

Table 1: Proteolytic Screening of Selected Bacterial Isolates on Skim Milk Agar

Isolate	Hydrolysis halo (mm)
A ₁	18.00 ± 0.04
B ₃	22.00 ± 0.025

Note: Values are mean ± SD from duplicate measurements (n = 2). The values represent the total diameter of the visible clearing zone on skim milk agar.



Figure 1: Proteolytic activity of A₁ and B₃ on skim milk agar. A₁ (left) showed the smaller halo zone, while B₃ (right) showed the larger halo zone

Morphological and Biochemical Characteristics of Selected Isolates

Both selected isolates were cream-coloured, Gram-positive, rod-shaped and motile and were positive for catalase, citrate utilisation, casein hydrolysis, gelatin liquefaction, glucose

fermentation and sucrose fermentation. A₁ was positive for Voges-Proskauer and starch hydrolysis but negative for oxidase and urease, whereas B₃ was positive for oxidase and urease but negative for Voges-Proskauer and starch hydrolysis.

Table 2: Morphological and Biochemical Characteristics of the Selected Isolates

Characteristic	A ₁ /K1	B ₃ /K2
Colour/pigment	Cream	Cream
Gram-stain reaction	Positive	Positive
Shape	Rod	Rod
Motility	Positive	Positive
Spore staining	Positive	Sub-terminal spores
Catalase	Positive	Positive
Oxidase	Negative	Positive
Urease	Negative	Positive
Citrate utilization	Positive	Positive
Indole test	Negative	Negative
Methyl red	Negative	Negative
Voges-Proskauer	Positive	Negative
Starch hydrolysis	Positive	Negative
Casein hydrolysis	Positive	Positive
Gelatin liquefaction	Positive	Positive
Glucose fermentation	Positive, no gas	Positive, no gas
Lactose fermentation	Negative	Negative
Sucrose fermentation	Positive, no gas	Positive, no gas

Molecular Identification of Selected Isolates

BLAST analysis identified isolate K1 as *Bacillus cereus* and isolate K2 as *Lysinibacillus fusiformis*. K1 had a sequence length of 1518 bp, 99.34% identity, 100% query coverage and

an E-value of 0.0 against accession GQ406846.1. K2 had a sequence length of 1516 bp, 99.93% identity, 100% query coverage and an E-value of 0.0 against accession OQ568519.1.

Table 3: Molecular Identification of Selected Bacterial Isolates

Isolate	Organism	Sequence length (bp)	Identity (%)	E-value	Query coverage (%)	Closest BLAST accession
A ₁ (K1)	<i>B. cereus</i>	1518	99.34	0.0	100	GQ406846.1
B ₃ (K2)	<i>L. fusiformis</i>	1516	99.93	0.0	100	OQ568519.1

Note: GQ406846.1 and OQ568519.1 are the accession numbers of the closest BLAST reference matches.

Phylogenetic Placement

The 16S rRNA cladogram placed A₁/K1 with *Bacillus cereus* reference sequences, including GQ406846.1, and B₃/K2 with

Lysinibacillus fusiformis reference sequences, including OQ568519.1.

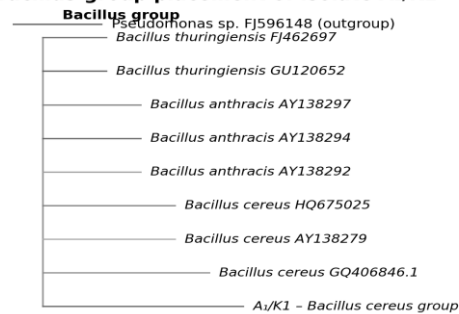
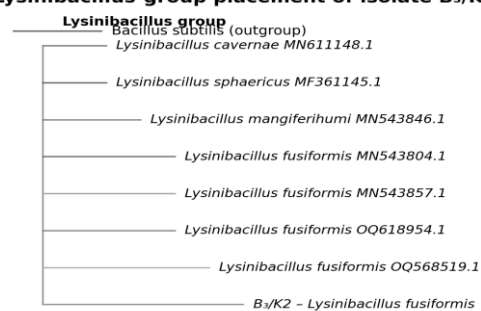
A. Bacillus-group placement of isolate A₁/K1**B. Lysinibacillus-group placement of isolate B₃/K2**

Figure 2: Cladogram showing the 16S rRNA placement of isolates A₁/K1 and B₃/K2 relative to their closest BLAST matches and selected related reference taxa.

Keratinase Production During Fermentation

Keratinase activity increased during the early and middle stages of fermentation and declined after the peak period in both isolates. A₁/K1 increased from 2.40 ± 0.11 U/mL on day

1 to 42.88 ± 1.71 U/mL on day 7 and declined to 17.44 ± 0.72 U/mL on day 14. B₃/K2 increased from 1.12 ± 0.06 U/mL on day 1 to 28.64 ± 1.12 U/mL on day 5 and declined to 14.84 ± 0.61 U/mL on day 14.

Table 4: Keratinase Activity of the Selected Isolates During Cattle Horn Fermentation (U/mL)

Isolate	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14
<i>B. cereus</i> (A ₁)	2.40 ± 0.11	15.60 ± 0.62	32.96 ± 1.24	42.88 ± 1.71	37.96 ± 1.48	25.60 ± 1.02	17.44 ± 0.72
<i>L. fusiformis</i> (B ₃)	1.12 ± 0.06	10.76 ± 0.44	28.64 ± 1.12	27.84 ± 1.09	23.24 ± 0.94	18.08 ± 0.76	14.84 ± 0.61

Note: Values are mean $\pm$ SD from three biological fermentation replicates; analytical enzyme activity measurements were performed in duplicate.

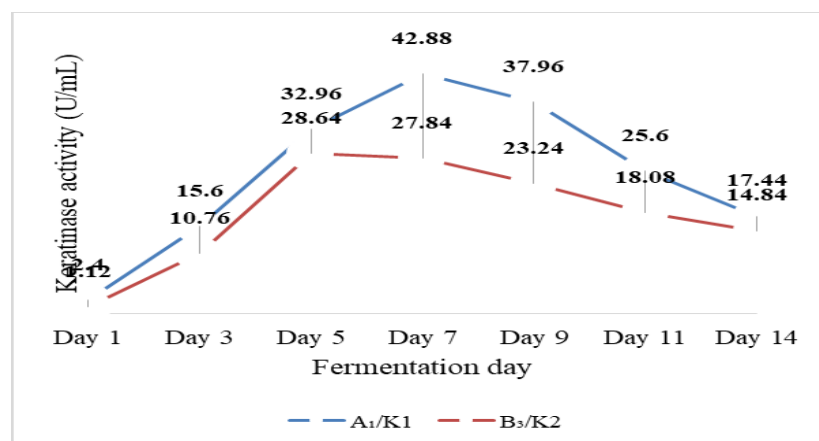


Figure 3: Keratinase activity of A₁/K1 and B₃/K2 during 14 days of cattle horn fermentation. Error bars represent standard deviation

Discussion

Because skim milk agar contains casein, the clearing halos produced by A₁ and B₃ demonstrate extracellular caseinolytic activity and are appropriate for preliminary proteolytic screening, but casein hydrolysis alone does not establish keratinase activity (de Menezes *et al.*, 2021; Deba *et al.*, 2023). The larger halo produced by B₃ therefore reflects greater casein hydrolysis under the screening conditions rather than a direct measurement of cattle-horn degradation (Deba *et al.*, 2023).

The Gram-positive, rod-shaped and spore-forming characteristics observed for the selected isolates are consistent with phenotypes reported for keratinolytic bacteria recovered from keratin-rich waste environments (Aktayeva *et al.*, 2022; Vema and Kumar, 2023). BLAST analysis identified K1 as *Bacillus cereus* with 99.34% identity and K2 as *Lysinibacillus fusiformis* with 99.93% identity. Although the high 16S rRNA similarity supports close taxonomic placement, 16S rRNA sequences may provide limited species-level discrimination within the *Bacillus cereus* group (Janda and Abbott, 2007; Liu *et al.*, 2015).

A₁/K1 reached its highest keratinase activity on day 7, whereas B₃/K2 reached its highest activity on day 5. Time-dependent increases followed by decreases in keratinase activity have also been reported in bacterial keratin fermentation systems (Lai *et al.*, 2023; Nnolim and Nwodo, 2020). Changes in nutrient availability, microbial growth phase and extracellular enzyme stability can contribute to declining activity after a production peak, although these variables were not measured directly in the present study (Lai *et al.*, 2023; Nnolim and Nwodo, 2020).

Keratinase activity values reported across studies are not directly comparable when substrate type, substrate pretreatment, incubation conditions, enzyme preparation, assay wavelength and unit definition differ, because keratinase assays remain poorly standardised across the literature (de Oliveira Martinez *et al.*, 2020). Feather-based studies, for example, have used different fermentation conditions and activity definitions from those applied to cattle horn in the present study (Bokveld *et al.*, 2021; Derhab *et al.*, 2024; Gerlicz *et al.*, 2024).

Activity in the present study was measured in crude cell-free supernatant by absorbance at 280 nm after incubation with cattle horn powder, and absorbance at 280 nm is influenced mainly by aromatic amino acids and other protein-derived material in the sample matrix (Reinmuth-Selzle *et al.*, 2022). Keratinase studies use several different assay systems, and direct substrate-loss measurements or complementary characterization of released products can strengthen evidence of keratin degradation (de Oliveira Martinez *et al.*, 2020). Therefore, the absorbance-based assay describes the time course of activity under the stated conditions but does not by itself quantify the mass of cattle horn degraded.

The repeated sampling design followed the same fermentation vessels through time, and the study reported descriptive means and standard deviations without inferential testing. The observed differences between A₁/K1 and B₃/K2 should therefore be interpreted as differences in the recorded time-course values rather than statistically established differences between isolates.

CONCLUSION

Six bacterial isolates were recovered from abattoir soil, and A₁ and B₃ were selected after producing measurable proteolytic clearing zones on skim milk agar. BLAST analysis identified K1 as *Bacillus cereus* and K2 as *Lysinibacillus fusiformis*. During cattle horn fermentation, K1

reached a maximum keratinase activity of 42.88 ± 1.71 U/mL on day 7, while K2 reached 28.64 ± 1.12 U/mL on day 5. These findings show that cattle horn powder supports keratinase activity by the selected isolates under the tested fermentation conditions.

Further studies should standardize inoculum density, validate the keratinase assay with appropriate controls and calibration, and confirm cattle horn degradation using direct substrate-loss measurements or characterization of released hydrolysis products. Independent fermentation experiments should also be used for inferential comparison of isolates, while optimization of pH, temperature, inoculum size, substrate concentration and incubation time can be undertaken after assay validation.

REFERENCES

- Aktayeva, S., Baltin, K., Kiribayeva, A., Akishev, Z., Silayev, D., Ramankulov, Y. and Khassenov, B. (2022). Isolation of *Bacillus* sp. A5.3 strain with keratinolytic activity. *Biology*, 11(2), 244. <https://doi.org/10.3390/biology11020244>
- Banasaz, S. and Ferraro, V. (2024). Keratin from animal by-products: Structure, characterization, extraction and application—A review. *Polymers*, 16(14), 1999. <https://doi.org/10.3390/polym16141999>
- Bokveld, A., Nnolim, N. E. and Nwodo, U. U. (2021). *Chryseobacterium aquifrigidense* FANN1 produced detergent-stable metallokeratinase and amino acids through the abasement of chicken feathers. *Frontiers in Bioengineering and Biotechnology*, 9, 720176. <https://doi.org/10.3389/fbioe.2021.720176>
- Das, S., Das, A., Das, N., Nath, T., Langthasa, M., Pandey, P., Kumar, V., Choure, K., Kumar, S. and Pandey, P. (2024). Harnessing the potential of microbial keratinases for bioconversion of keratin waste. *Environmental Science and Pollution Research*, 31(46), 57478–57507. <https://doi.org/10.1007/s11356-024-34233-6>
- de Menezes, C. L. A., Santos, R. D. C., Santos, M. V., Boscolo, M., da Silva, R., Gomes, E. and da Silva, R. R. (2021). Industrial sustainability of microbial keratinases: Production and potential applications. *World Journal of Microbiology and Biotechnology*, 37(5), 86. <https://doi.org/10.1007/s11274-021-03052-z>
- de Oliveira Martinez, J. P., Cai, G., Nachtschatt, M., Navone, L., Zhang, Z., Robins, K. and Speight, R. (2020). Challenges and opportunities in identifying and characterising keratinases for value-added peptide production. *Catalysts*, 10(2), 184. <https://doi.org/10.3390/catal10020184>
- Deba, F., Nelofer, R. and Irfan, M. (2023). Isolation, identification, and screening of keratinase producing bacteria from soil and production optimization using feather waste as substrate. *Punjab University Journal of Zoology*, 38(1), 109–118. <https://doi.org/10.17582/journal.pujz/2023.38.1.109.118>
- Derhab, N., Mabrouk, M. E. M., El-Metwally, M. M. and Mohammed, Y. M. M. (2024). Thermostable keratinase from *Bacillus cereus* L10: Optimization and some potential biotechnological applications. *Biomass Conversion and Biorefinery*, 14, 29757–29773. <https://doi.org/10.1007/s13399-023-04887-2>

- Gerlicz, W. A., Sypka, M., Jodłowska, I. and Białkowska, A. M. (2024). Isolation, selection, and identification of keratinolytic bacteria for green management of keratin waste. *Molecules*, 29(14), 3380. <https://doi.org/10.3390/molecules29143380>
- Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761–2764. <https://doi.org/10.1128/JCM.01228-07>
- Lai, Y., Wu, X., Zheng, X., Li, W. and Wang, L. (2023). Insights into the keratin efficient degradation mechanism mediated by *Bacillus* sp. CN2 based on integrating functional degradomics. *Biotechnology for Biofuels and Bioproducts*, 16, 59. <https://doi.org/10.1186/s13068-023-02308-0>
- Liu, Y., Lai, Q., Göker, M., Meier-Kolthoff, J. P., Wang, M., Sun, Y., Wang, L. and Shao, Z. (2015). Genomic insights into the taxonomic status of the *Bacillus cereus* group. *Scientific Reports*, 5, 14082. <https://doi.org/10.1038/srep14082>
- Martínez, M. G. T., Luís, J. and Bolaños, N. (2023). *Lysinibacillus fusiformis*: A novel fibrolytic native strain from the rumen microbiome that increases in vitro digestibility of central agricultural residues. *Journal of Applied Animal Research*, 51(1), 658–668. <https://doi.org/10.1080/09712119.2023.2269227>
- Mpaka, L., Nnolim, N. E. and Nwodo, U. U. (2025). Microbial keratinolysis: Eco-friendly valorisation of keratinous waste into functional peptides. *Microorganisms*, 13(10), 2270. <https://doi.org/10.3390/microorganisms13102270>
- Nnolim, N. E. and Nwodo, U. U. (2020). *Bacillus* sp. CSK2 produced thermostable alkaline keratinase using agro-wastes: Keratinolytic enzyme characterization. *BMC Biotechnology*, 20(1), 65. <https://doi.org/10.1186/s12896-020-00659-2>
- Rahimnahal, S., Meimandipour, A., Fayazi, J., Karkhane, A. A., Shamsara, M., Beigi Nassiri, M., Mirzaei, H., Hamblin, M. R., Tarrahimofrad, H., Bakherad, H., Zamani, J. and Mohammadi, Y. (2023). Biochemical and molecular characterization of novel keratinolytic protease from *Bacillus licheniformis* (KRLr1). *Frontiers in Microbiology*, 14, 1132760. <https://doi.org/10.3389/fmicb.2023.1132760>
- Reinmuth-Selzle, K., Tchpilov, T., Backes, A. T., Tscheuschner, G., Tang, K., Ziegler, K., Lucas, K., Pöschl, U., Fröhlich-Nowoisky, J. and Weller, M. G. (2022). Determination of the protein content of complex samples by aromatic amino acid analysis, liquid chromatography-UV absorbance, and colorimetry. *Analytical and Bioanalytical Chemistry*, 414(15), 4457–4470. <https://doi.org/10.1007/s00216-022-03910-1>
- Sypka, M., Jodłowska, I. and Białkowska, A. M. (2021). Keratinases as versatile enzymatic tools for sustainable development. *Biomolecules*, 11(12), 1900. <https://doi.org/10.3390/biom11121900>
- Vema, R. and Kumar, V. (2023). Isolation and characterization of keratinolytic bacteria from poultry waste soils of Himachal Pradesh. *Journal of Applied Biology and Biotechnology*, 11(3), 172–179. <https://doi.org/10.7324/JABB.2023.112620>

