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Molecular identification and phylogenetic profiling of keratinolytic bacteria isolated from goose and chicken farms in a cold region of Türkiye

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Abstract

The aim of this study was to investigate bacterial diversity in certain goose and poultry farms in Kars, Türkiye a cold climate zone. Feather waste samples rich in organic compounds were collectedfrom multiple farm locations and evaluated for bacterial diversity. A total of 27 bacteria were isolated. 16S rRNA gene sequencing, morphological, physiological, biochemical and molecular techniques were used. Isolates included *Escherichia coli*, *Ralstonia picketti*, *Pantoea agglomerans*, *Kosakonia cowanii*, *Bacillus licheniformis*, *Bacillus zhangzhouensis*, *Bacillus pumilus*, *Exiguobacterium sibiricum*, *Exiguobacterium artemiae*, *Macrococcus caseolyticus*, *Mammaliicoccus vitulinus*, *Mammaliicoccus sciuri* and *Rothia nasisuis*. Most of the isolates are positive for characteristics such as spore formation, motility and catalase activity. These isolates also showed adaptation to nutrient-rich and varying temperature parameters. Molecular identification results confirmed the results of phylogenetic analyses, showing strong bootstrap support. The identification of resistant bacteria in these keratin-rich regions plays an important role in the control of poultry waste management. These bacteria, which are desirable for biotechnological applications, are also importance in enzyme studies. This study provides valuable information on the functional diversity of microbial communities in goose and poultry farming. It draws attention to the importance of microbial enzymes in the disposal of keratin wastes.

Keywords: 16S rRNA, Sequence, Microorganisms, Keratinase, Phylogenetic, Poultry and goose farm microbiota

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INTRODUCTION

Poultry farming is a primary means of meeting the global demand for animal protein (Josephson, 2020). Goose and chicken breeding, which come to mind first in poultry farming, contribute to the rural economy in terms of both meat and egg trade (Gilbert, Xiao, & Robinson, 2017). Kars and its surroundings provide a favourable environment for goose breeding due to its geographical conditions and cold climate. Microbial organisms are one of the most important factors affecting production efficiency in goose and poultry farms (Akhtar, Shafiq, & Ali, 2022). Poultry environments are home to many bacteria (Xu et al., 2022). These bacteria can include both pathogenic and beneficial species. Determination of the diversity of bacteria is very important in animal breeding (Wang & Jin, 2019). Bacteria can be found in large quantities in the gut microbiota of poultry and in the poultry house. Beneficial bacteria contribute to the correct functioning of the digestive system of poultry (Rychlik, 2020). Pathogenic bacteria, on the other hand, can cause diseases leading to poultry mortality in a short time. Especially bacteria such as *Salmonella*, *E. coli* and *Staphylococcus aureus* are among the most undesirable pathogens in poultry farming (Geletu, Usmael, & Ibrahim, 2022). For this reason, determination of bacterial diversity is very valuable for the continuity of animal husbandry, especially in rural areas, both for the protection of animal health and the prevention of zoonotic diseases.

In recent years, molecular biology techniques have been frequently preferred in the determination of microbial diversity because of more precise results. 16S rRNA gene sequencing and metagenomic analyses are among the most widely used methods by researchers (Clarridge Iii, 2004). In addition to these, biochemical tests are also used effectively in the identification of bacteria. These techniques can reveal detailed analyses on bacterial diversity. Identification of bacteria isolated from goose and poultry farms in and around Kars will provide a scientific contribution to the livestock farming activities in the region. It will be inevitable to take necessary precautions against pathogens that may be present in these environments and to get more effective results in farm management.

Bacteria with keratinolytic activity play an important role in the biological transformation of keratin-rich organic materials, especially poultry waste. These bacteria are able to biodegrade environmentally harmful wastes by hydrolyzing difficult-to-degrade proteins such as keratin with keratinase enzymes and in this process, valuable by-products (amino acids, peptides) are formed. Thanks to these properties, they are used in various industrial applications such as recycling of agricultural wastes, feed additive production and biological dehairing in the leather industry. They also offer important biotechnological opportunities to reduce environmental pollution caused by the indiscriminate release of keratin wastes into nature and to make waste management more sustainable (Akhter, Wal Marzan, Akter, & Shimizu, 2020; Vikash, Kamini, Ponesakki, & Anandasadagopan, 2025). In recent years, keratinolytic bacteria have emerged as sustainable and environmentally friendly biotechnological tools, especially for the biodegradation of poultry feather waste. The potential of these bacteria to convert organic wastes into valuable by-products through keratinase production has important applications in industrial biotechnology and environmental bioremediation (Gerlicz, Sypka, Jodłowska, & Białkowska, 2024; Moktip et al., 2025).

This study targeted the microbial diversity isolated from goose and chicken farms in Kars and its surroundings. The obtained findings are intended to contribute to the measures to be taken in the protection of animal health, targeted antibiotic selection and the direction in which farm hygiene should be progressed and improved. In addition, the results of this study can help to understand the microbial risks and what measures can be taken against these risks.

MATERIALS AND METHODS

Biological Samples

The biological material of this study comprises bacteria isolated from feather waste and environmental samples collected from goose and chicken farms in Kars during the breeding season. The spatial distribution of the sampling sites is illustrated in Figure 1. Samples were meticulously gathered from areas with dense feather waste and organic materials in the vicinity of farmyards. Sterile 2-litre glass jars sealed with aluminium foil to prevent exposure to sunlight were used for sample collection. Coordinates of each sampling site were recorded for documentation and reproducibility. In each location, samples were collected at consistent intervals, ensuring representation from different sections of the farms. A portable digital device (Model No. AZ 8685, Taiwan) was used to measure the temperature and pH of the samples at the time of collection. The probe was inserted 5 cm into the substrate. Immediately following collection, samples were either kept at +4°C for processing or transferred into sterile tubes and subjected to serial dilution (10^{-1} to 10^{-6}) with physiological saline (NaCl 0.85 g per 100 mL distilled water) for the purpose of bacterial isolation (Ulucay, Gormez, & Ozic, 2022b).

A 0.1 mL aliquot from each dilution was plated on Luria Bertani (LB) agar (Sigma-Aldrich), and the plates were incubated for 24 to 72 hours at temperatures ranging from 35 to 45 ºC. The colonies with distinct morphologies were selected for further study. Feather waste, which is abundant in the sampled farms, was the main source of keratin-degrading bacteria, showing potential for keratinase enzyme production, an important step for industrial applications of these isolates (Gerlicz et al., 2024).

Identification of bacteria isolates

Morphological Characteristics

Examinations for morphological features and motility patterns are carried out on a light microscope (BS-2030T, Bestscope) (Silva-de la Fuente et al., 2021). Gram staining and endospore imaging were performed to determine cell wall characteristics and spore formation, while motility was assessed using standard wet mount techniques described in the literature (Turenne, Snyder, & Alexander, 2015).

Motility

The motility tests, cell morphology, and endospore production of freshly cultured (24-h-old) bacterial samples were observed by microscopy (Bestscope BLM-280 LCD, China). These observations were useful for preliminary classification and differentiation of bacterial groups prior to molecular identification (Ulucay, Gormez, & Ozic, 2022a)

Gram and Endospore Staining

Using a sterile loop, colonies were transferred to one side of a clean microscope slide and resuspended in physiological saline. The slides were air-dried and heat-fixed, then immersed in crystal violet solution for 2 minutes, followed by a water rinse. Lugol's iodine solution was applied for 1 minute to enhance staining, and after another rinse and drying step, 96% alcohol was used to decolorize for 15-20 seconds. After a final water rinse and drying, fuchsin dye was added for 30 seconds to complete the staining. Dried slides were soaked in blotting paper

before being observed by mounting under an immersion lens on a microscope using immersion oil. Bacteria stained blue or purple were identified as gram-positive, while those with a pink color were classified as gram-negative (Ulucay et al., 2022b).

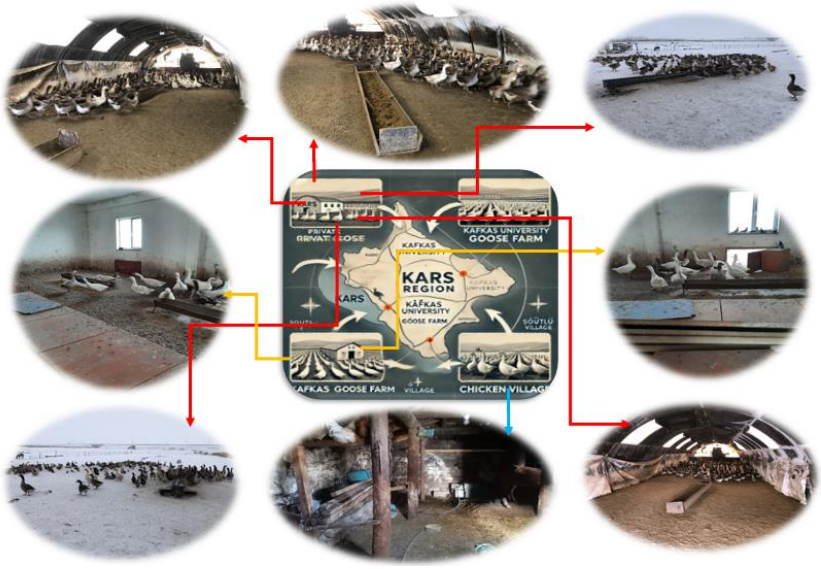

Figure 1. Areas highlighted with red arrows indicate the Kars Private Goose Farm, those marked with yellow arrows represent the Kafkas University Goose and Chicken Farm, and the areas identified by blue arrows depict the Söğütlü Village Goose and Chicken Farm.

Endospore staining was performed on overnight cultures grown on LB agar. Colonies were subsequently transferred to glass microscope slides using a sterile loop. Slides with dried preparation were stained with carbol fuschsin for 5 minutes then rinsed and treated with 10% nitric acid for 10 seconds. Methylene blue was then reacted against the counter cells for 2 minutes, washed, and allow to dry by air. The images were set for observation with an immersion oil under the microscope (Beveridge, Lawrence, & Murray, 2007).

Physiological Properties

The physiological characteristics of the isolates, including their responses to varying temperatures, pH levels, and sodium chloride (NaCl) concentrations, were examined across different growth stages under aerobic conditions (Oztas Gulmus & Gormez, 2020). Incubation temperatures ranged from 36°C to 45°C, and growth was monitored at pH levels from 4.0 to 12.0 in increments of 1.0. To assess the effect of NaCl concentration, the growth medium was supplemented with NaCl up to 10% (w/v). Optical density was the method used for quantifying bacterial growth to measure by spectrophotometer (GENESYS, Thermo Scientific) at 600 nm (Md Sidek et al., 2018).

Genomic DNA Isolation

Genomic DNA analyses of bacteria were carried out by modifying the classical phenol: chloroform: isoamylalcohol method. Genomic DNA samples were stored at -80°C until the 16S rRNA locales were dissected (Sambrook, 1989).

Sequencing of 16S rRNA

Subjected to a modified classical protocol with phenol, chloroform, and isoamyl alcohol, total genomic DNA was extracted for further molecular identification using 16S rRNA sequencing (Nikunjkumar, 2012). To amplify the 16S rRNA gene, we used the thermal cycler (Gene Pro, Bioer Technology Co., Ltd, TC-E-96G) as described before (Meena & Baranwal, 2016). The amplification of the region of interest in DNA was accomplished with universal primers: the forward primer 27F and the reverse primer 1385R (Ulucay et al., 2022b). The reactions for PCR were carried out in a total volume of 25 µL which included 10X PCR buffer (Applied Biosystems, Roche), 25 µM magnesium chloride, 25 mM dNTPs, 25 pmol of each primer, and 5 units/µL of Taq DNA polymerase (Applied Biosystems; Table 1). The PCR amplifying cycle was done a total of 40 times, one would have been run through ordinary denaturation, annealing at 55°C, and extension steps, with finally, a final extension lasting for 10 minutes at 72°C.

A 1% agarose gel was utilized in the electrophoretic process for PCR product analysis. The purified PCR products were cloned into vectors and subsequently sequenced. The resultant sequence data were analyzed against GenBank and deposited in the NCBI GenBank sequence database.

Table 1. Basic PCR Components

Component	Quantities
Taq Polymerase (5U/µL)	0,25 µL
10x Buffer PCR	2,5 µL
$MgCl_2$ (25 mM)	2 µL
Primer 27 F (25pmol/ µL)	2,5 µL
Primer 1385 R (25pmol/ µL)	2,5 µL
DNA	1 µL
dNTP (25mM)	1,5 µL
ddH_2O	12,75 µL

Phylogenetic

Phylogenetic status among the identified isolates was inferred using the Neighbour-Joining algorithm implemented in MEGA11 software (Tamura, Stecher, & Kumar, 2021). Type strains and outgroups were included in the analysis to ensure the appropriate taxonomic configuration of each isolate based on 16S rRNA gene sequences. The robustness of the resulting phylogenetic tree was assessed through 1000 bootstrap replicates with branch support values indicating the frequency of clustering between resampled datasets (Felsenstein, 1992). Evolutionary distances between sequences were calculated using the p-distance model, allowing the assessment of genetic divergence between lineages. The final tree structure was organised to reflect both evolutionary closeness and taxonomic resolution (Nei & Kumar, 2000).

RESULTS AND DISCUSSION

Isolate Screening and Classification

A total of 27 bacterial isolates were identified from goose and poultry farms in Kars city centre and Söğütlü village. In the initial isolation stages, the ability of bacteria to grow under aerobic conditions was investigated.

The isolates able to grow at high temperatures (50-60 °C) were selected. Colonies with different morphological characteristics were selected and grown on feather meal agar and casein containing media. Preliminary screening showed that some isolates produced extracellular keratinase activity in the medium, causing clear zones to appear in the medium. The diameter of these zones varied, reflecting differences in enzymatic diffusion and degradation potential.

The isolates were evaluated for gram staining, endospore formation, motility, catalase and oxidase activities and tolerance to varying NaCl concentrations and pH ranges (Figure 2 and Table 2). The majority of the bacterial isolates obtained were gram-negative, motile and catalase-positive. Strains belonging to the genus *Bacillus* generally showed sporulation capacity. 16S rRNA gene amplification and sequencing techniques were used for the identification of the isolates. The sequences obtained as a result of sequencing were analyzed using BLAST in the NCBI database and taxonomic comparison was made. The genera identified included *Bacillus*, *Kosakonia*, *Exiguobacterium*, *Pantoea*, *Ralstonia*, *Escherichia*, *Rothia*, *Macrococcus* and *Mammaliicoccus*. Phylogenetic analysis grouped these isolates into distinct clusters with high bootstrap support, highlighting their evolutionary relationships (Figure 5). These findings provide the identification of bacterial cultures with keratinolytic potential in poultry raised under cold climate conditions in Kars region. The predominance of both spore-forming and facultatively anaerobic species underlines the adaptability and resistance of these isolates under harsh ecological conditions.

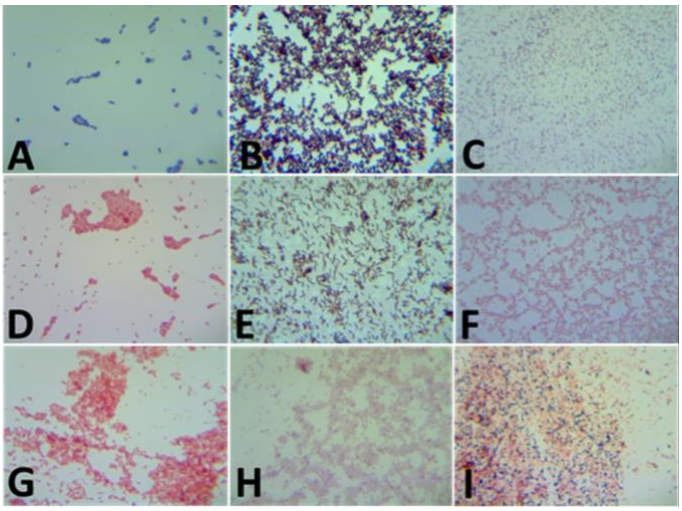

Figure 2. Gram staining of the isolates. **A:** A4, **B:** A5, **C:** A8, **D:** B3, **E:** C10, **F:** C12, **G:** D2, **H:** D5 and **I:** D6

Table 2. Phenotypic and molecular characterization of bacterial isolates recovered from goose and poultry farm samples.

Isolate Code	GenBank ID	Identification	Location	Temp./ T_{opt} (ºC)	pH	NaCl (%)	Anaerobic growth	Chain	Movement	Endospore	Gram Test
A1	PQ632386	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
A2	PQ632387	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
A4	PQ632388	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
A6	PQ632389	*Macrococcus caseolyticus*	Center	36-95/55	5-7	≤ 7	+	-	-	-	+
A8	PQ632390	*Escherichia coli*	Center	36-95/55	5-7	≤ 7	+	-	+	-	-
B1	PQ632391	*Mammaliicoccus vitulinus*	Kafkas	36-95/55	5-7	≤ 7	+	-	-	-	+
B2	PQ632392	*Escherichia coli*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
B3	PQ632393	*Rothia nasisuis*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	-	+
B4	PQ632394	*Mammaliicoccus sciuri*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	+
B5	PQ632395	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
B6	PQ632396	*Escherichia coli*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
B8	PQ632397	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C1	PQ632398	*Bacillus zhangzhouensis*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	+	+
C2	PQ632399	*Exiguobacterium sibiricum*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	+
C3	PQ632400	*Bacillus pumilus*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	+	+
C4	PQ632401	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C5	PQ632402	*Kosakonia cowanii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C8	PQ632403	*Kosakonia cowanii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C9	PQ632404	*Exiguobacterium artemiae*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	+
C10	PQ632405	*Mammaliicoccus vitulinus*	Kafkas	36-95/55	5-7	≤ 7	+	-	-	-	+
C11	PQ632406	*Ralstonia* sp.	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C12	PQ632407	*Ralstonia pickettii*	Kafkas	36-95/55	5-7	≤ 7	+	-	+	-	-
C13	PQ632408	*Bacillus licheniformis*	Kafkas	36-95/55	5-7	≤ 7	+	+	+	+	+
D2	PQ632409	*Pantoea agglomerans*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-
D4	PQ632410	*Ralstonia pickettii*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-
D5	PQ632411	*Pantoea agglomerans*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-
D6	PQ632412	*Pantoea agglomerans*	Söğütlü	36-95/55	5-7	≤ 7	+	-	+	-	-

16S rRNA Gene Regions of Isolates

Genomic DNA from bacterial isolates was visualised by electrophoresis gel verification (Figure 3). DNA bands that appeared clear were considered indicative of a quality DNA isolate for further analyses. Repeated isolation studies were performed for genomic DNAs that could not be fully visualised. All universally agreed upon primers, including those targeting conserved regions, were enlisted to magnify the 16S RNA gene. Agarose gel electrophoresis (Figure 4) confirmed about 1300 to 1500 base pairs. The gel purified products were subjected to sequencing analysis. The amplicons obtained were compared with the NCBI database with the use of BLASTn to identify the similarity percentages and name the isolates. Isolates successfully identified at the genus or species level were assigned accession numbers through the NCBI system (Table 2). Many isolates are commonly found in organic-rich environments. These isolates showed high sequence similarity to *E. coli*, *R. pickettii* and *P. agglomerans*. In addition to these common species, several thermotolerant or endospore-forming genera such as *Bacillus* and *Exiguobacterium* have been identified. GenBank accession numbers and corresponding isolate codes are presented in Table 2.

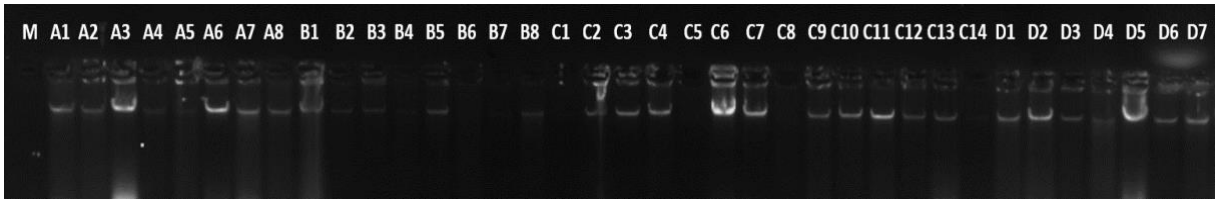

Figure 3. Genomic DNA extraction profiles of bacterial isolates recovered from goose and poultry farm environments. M: DNA ladder; lanes A1–D7 represent isolate codes.

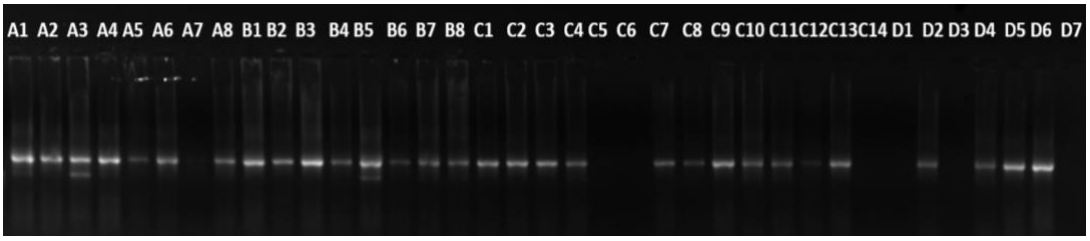

Figure 4. PCR amplification of 16S rRNA gene regions using universal primers. Amplicons are approximately 1500 bp. M: DNA ladder; lanes A1–D7 correspond to bacterial isolates. (GenBank PQ632386 - PQ632412)

Taxonomic Distribution of the Isolates

The bar chart shown in Figure 5 displays the relative abundance of the identified genera. Among the above-mentioned anaerobes belong facultative anaerobes like *E. coli* and *R. pickettii*, while also a group of well-represented sporulating genera such as *Bacillus* and *Exiguobacterium*. Findings like these indicate adaptation by microbes to environments that are high in nutrients and thermally variable, such as farms.

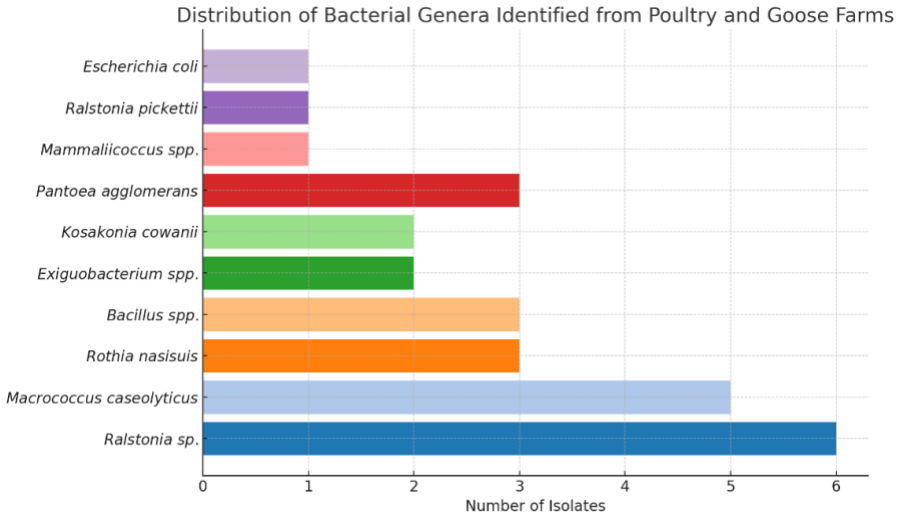

Figure 5. Distribution of bacterial genera identified from goose and poultry farm environments. The bar chart shows the number of isolates corresponding to each genus based on 16S rRNA gene sequencing results.

16s-Based Phylogeny

A phylogenetic tree was produced by the Neighbour-Joining method using the 16S rRNA gene sequences of the 27 bacteria isolated from the poultry area. The phylogenetic tree was rooted with *Bacteroides fragilis* as the outgroup and reference strains were included for the ingroup (Figure 6).

The study identified a total of 9 major clades, each of them strongly supported by bootstrap values, and revealed different relationships in terms of evolution among the isolates. One of the major clusters contained *E. coli* strains A1, A2, A4, B2, A8, B6 and grouped it closely to *E. coli* strain U 5/41 with bootstrap values falling within 66-97. This uniformity supports the reliability of their identification. Another clustered clade which demonstrates a quite close phylogenetic affinity between members of the *Enterobacteriaceae* is that with *K. cowanii* (C5, C8) and *P. agglomerans* (D5, D6); whereas, *R. pickettii* isolates (B5, B8, C4, C12, D4), which together with *R. pickettii* RAL01, formed a coherent cluster that was supported by bootstrap values ranging between 55 and 96, indicating a very strong monophyletic origin.

Spore-forming isolates such as *B. pumilus*, *B. lichenformis* and *B. zhangzhouensis* were clearly separated from other gram-positive bacteria such as *E.sibiricum*, *M. vitulinus* and *M. caseolyticus*. Interestingly, *Exiguobacterium* isolates (C2, C9) closely clustered with *E. artemiae*, suggesting possible environmental adaptation to stress-resistant niches.

The overall structure of the tree illustrates the results of molecular identification and ecological variation among the isolates with isolate consistency. The presence of distinct races, such as facultative anaerobes, thermotolerance bacilli and environmental opportunists, highlights the wide variety of bacterial taxa that thrive in habitats dependent on geese and poultry livestock.

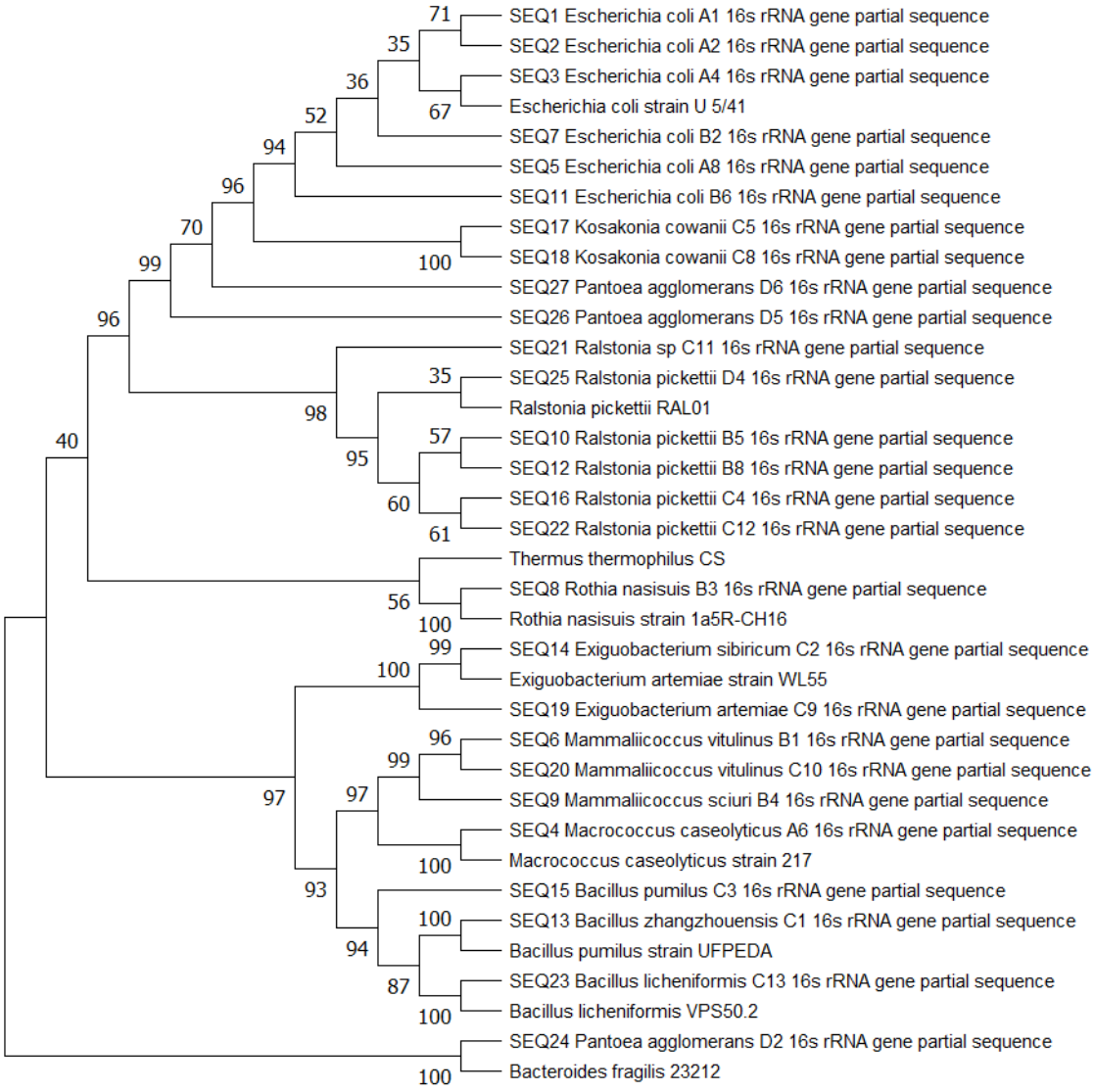

Figure 6. A molecular phylogenetic tree based on 16S rRNA was constructed by the neighbor-joining method with 1000 bootstrap replicates using *Bacteroides fragilis* 23212 as the outgroup.

This study provides information on microbial bacterial diversity obtained from goose and chicken farms in Kars, one of the coldest provinces of Turkey. On the basis of the results of these morphological, biochemical, and molecular characterizations, including the 16S rRNA gene sequence analysis, 27 isolates belonging to the genera *Escherichia*, *Ralstonia*, *Pantoea*, *Kosakonia*, *Exiguobacterium*, *Bacillus*, *Rothia*, *Macrococcus* and *Mammaliicoccus* were identified. These outcomes yield at least the essential results in the context of bacterial diversity encountered in agricultural ecosystems characterized by the accumulation of organic waste.

Species such as *E. coli*, *R.pickettii* and *P. agglomerans* in the study area, which is contaminated with faeces and rich in organic compounds, are consistent with other reported studies (Althouse & Lu, 2005; Charnock, 2004; Mokoena, 2013; Väisänen, Weber, Bennasar, Rainey, & Busse, 1998). *E. coli*, a facultative anaerobe frequently encountered in the gut microbiota of poultry, has been reported similarly in previous studies (Adil & Magray, 2012; Peng et al., 2021). However, the presence of this bacterium in external environmental samples in the study area increases the risk of contamination. Due to their antibiotic resistance, the isolates provide an excellent opportunity for the spread of potential zoonoses (Dafale, Srivastava, & Purohit, 2020). *R. pickettii* can naturally resist many antibiotics; therefore, it is frequently isolated from soil and water-associated environments. This species is also known as an opportunistic pathogen (Ryan & Adley, 2013).

The frequent isolation of *R. pickettii* during this study points to its ability to readily adapt to the nutrient-rich feather waste. Furthermore, even though it is another definition of a species found on plant surfaces, *P. agglomerans* can also adapt to poultry settings (Dutkiewicz, Mackiewicz, Lemieszek, Golec, & Milanowski, 2016; Holmström et al., 2025). In humans and animals, *P. agglomerans* can cause infections (Yu et al., 2025). In addition to their pathogenic properties, these genera contribute to the nutrient cycle and are involved in the hydrolysis of keratin.

Thermotolerant and spore-forming bacteria such as *B. licheniformis*, *B. pumilus* and *E. sibiricum* usually exist in protein-rich environments. The habitats of poultry are also favourable environments for these species. *Bacillus* species that can synthesize enzymes like keratinase and protease have drawn attention in the area of protein microbial hydrolysis (Areche et al., 2025; de Oliveira Sousa et al., 2025; Vikash et al., 2025). For instance, *B. licheniformis* degrades biological wastes that contain keratin. It also converts keratin wastes into hydrolysates rich in amino acids (Tadevosyan, Margaryan, & Panosyan, 2025). Similarly, *Exiguobacterium* species capable of keratin hydrolysis can adapt to both cold and hot environments. This adaptability makes them important for industrial biotechnology (Galván, Alonso-Reyes, & Albarracín, 2025).

The phylogenetic analysis results grouped the isolates with reference strains at the species level with high bootstrap support values and taxonomically correctly positioned. *Enterobacteriaceae* family members such as *K. cowanii* and *P. agglomerans* had similarities in phylogenetics (Berinson et al., 2020). Example separation of *Bacillus* species from gram-positive cocci (*Macrococcus*, *Mammaliicoccus*) demonstrates selection pressures in the environment leading to evolutionary divergence. When observed ecologically, factors such as temperature, oxygen and abundant keratinous nutrient media influence microbial diversity and adaptation. Similar occurrences of keratinolytic bacteria have been observed in environments like compost, feather waste, chicken manure, and slaughterhouses (Ningthoujam et al., 2018).

The findings suggest potential for future industrial applications. From the perspective of environmental sustainability and economic benefit, the transformation of keratinous wastes (mainly feathers) by microorganisms assumes great significance. Traditional disposal methods (like incineration or dumping) cause environmental devastation and create costs. However, using local microbes that are able to produce keratinase can help in ameliorating these processes (Akhter et al., 2020). The presence of rare species such as *M. caseolyticus* and *M. vitulinus*, whose keratinolytic abilities have not been clearly revealed, also makes the study interesting. Comparative genomic analyses show that these genera are active in antimicrobial peptide production.

CONCLUSION

This study revealed that keratinolytic bacteria isolated from goose and poultry farms have an important potential in the biological transformation of feather waste. Especially spore-forming and thermotolerant species were found to be effective in these processes. These bacteria are valuable biocatalysts for sustainable waste management and environmentally friendly biotechnological applications. In future studies, it is recommended to investigate the keratinase production potential of these isolates in detail and to carry out studies for industrial scale applications.

Future studies should focus on examining keratinase activity in more detail and investigating how it can be used more advantageously in industrial areas.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text and Table are original and that they have not been published before

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