ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation, Identification And Characterization Of Keratinase Producing Bacteria From Poultry Farms.

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ABSTRACT

To meet consumer demand, the global poultry business is constantly expanding; hence it is crucial to ensure the biosafety of the poultry farms. In poultry farms, the main sources of bacterial development, increased antibiotic resistance, and environmental dispersion may be poultry feed, litter, and water. The focus of this research is on the identification and characterization of bacterial pathogens found in commercial poultry farms, drinking water samples, feed samples, and litter samples. The 100 samples were gathered from chicken farms in different districts of Telangana (jagtial, Sircilla, Karimnagar, Nizamabad) and transported to the lab for additional examination. Serial dilution was used to analyse the samples, which were then examined for colony morphology, microscopic features, and the biochemical traits of the bacteria *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp., *Shigella* spp., *Bacillus* spp., *staphylococcus* spp., and *Streptococcus* spp. The results of this research indicate that the main sources of bacterial infections are chicken feed, drinking water samples and poultry litter.
Keywords: Keratin, Keratinase, Microbes, Poultry, biochemical test.

https://doi.org/10.33887/rjpbcs/2026.17.1.3

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ISSN: 0975-8585

INTRODUCTION

Humans are at risk of contracting food-borne illnesses because of contaminated poultry products caused by bacterial infections. Food-related illnesses caused by germs like *Salmonella* spp. and *E. coli* are spread through poultry meat [1]. The elements that affect the contagious transmission of bacterial infections must be taken into consideration to prevent them from emerging from poultry habitats. Poultry feed (starters, growers, finishers, and layers) and drinking water from poultry farms are contaminated during processing [2], storage while adding the raw material ingredients, and ultimately during exposure of the product to the environmental microbes [3,4]. The primary worry of industrialized nations is the safety of drinking water and chicken feed in poultry farms. All birds' basic needs are food and water, and contaminated feed results in significant economic loss [5]. The abundance of different bacteria in chicken diets depends on several variables, including the nutrient makeup of the feed, pH, oxygen tension, and water activity. According to Aisha Ali [6] and [7], microbial proliferation can reduce feed quality by replacing the nutrients in the feed, and damage done during feed processing or the creation of toxins has a detrimental influence on animal health [8]. The safe use of poultry products increases the length of time that must pass before microbiological contaminants are assessed and exposed, which causes the customer undue harm [9]. According to Osei et al. [10], the material used to create poultry beds, feather waste, manure, and leftover feed are the main sources of litter utilized by poultry. Bacterial infections can spread through leftover poultry.

The expanding population and the commerce of animals are under increasing health and ecological hazard [11]. Numerous illnesses in poultry are caused by bacterial infections such *Escherichia coli, Staphylococcus,* and *Bacillus* species. Although research is being done to repurpose chicken waste material for agricultural and feed additives for other animals [12], efficient poultry waste disposal practices are not currently in practice in developing countries [13,14]. The use of antibiotics in animal feed at low doses for extended periods of time may contribute to microbial resistance. Due to the risk of resistance genes propagating to the environment, it is possible for resistant bacteria to enter sewage systems through chicken waste [15,16]. As a result, it was suggested that the current research work isolate, characterize, and identify the isolated pathogenic bacteria linked to poultry as a source of the potential contamination in poultry farms [17]. Keratinases (EC.3.4.99.11) belong to the group of serine proteases capable of degrading keratin. It is an extracellular enzyme produced in a medium containing keratinous substrate such as feathers and hair. Keratinases have applications in traditional industrial sectors including feed, detergent, medicine, cosmetics and leather manufacturers, they also find application in more recent fields such as prion degradation for treatment of the dreaded mad cow disease, biodegradable plastic manufacture and feather meal production. Hence present study focuses on the production of enzyme keratinase. Because of the numerous potential uses of keratinases, study was undertaken to screen a bacterium that produces active keratinase.

MATERIALS AND METHODS

Sample Collections

Six chicken farms in the Tamil Nadu state of India's jagtial, Sircilla, Karimnagar, Nizamabad districts provided samples of poultry litter, poultry water, and commercial feed. Litter samples from chicken farms were chosen at random, and feed and water samples were gathered directly from the chicken farms' feeding operations. In total, 100 samples were acquired from each farm, including at least 6 litter, 6 feed and 6 water samples. The samples were taken in a sterile bag and container, and they were clearly labelled.

Analysis of Samples

The Mathan et al. [18] protocol was followed for the isolation and subsequent identification of the bacterial pathogens. 1 g of poultry litter, 1 g of chicken feed, and 1 ml of water sample were inoculated into a 100 ml nutritional broth and then incubated for an additional 24 hours at 37°C. All the samples that were obtained underwent a total bacterial count on nutrient agar plates before being serially diluted at concentrations of 1 in 10. After 24 hours of incubation, the colony formation is expressed in CFU/mg using a colony counter [19].

Characterization pf bacterial isolates

Gram Staining and Microscopy

The isolated bacterial cultures were characterized by morphological and biochemical activity analysis. The cultures were examined via gram staining and microscopy. Further the gram negative and positive isolated bacterial cultures were further examined under microscope. After 24 hours of incubation, the colonial morphology of a bacterial culture including its size, shape, colour, and texture was inoculated into different media [20,21].

Biochemical tests

Bio-chemical tests such as the catalase test, oxidase test, citrate utilisation test, Methyl red test, Indole test, Voges-proskauer test, triple sugar iron test, and carbohydrate fermentation tests were carried out using isolated bacterial strains that displayed distinctive colony morphology [22,23]. The selected bacterial cultures were allowed for large scale growth and culture was centrifuged to collect the supernatant. The keratinase enzyme is an extracellular enzyme where bacteria excrete outside of plasma membrane. The collected supernatant was used for keratinase enzyme activity via keratinase assay.

Keratinase Assay

The isolated potent strain (KP9) was transferred to 25 ml of the seed medium containing (g/L) peptone, 5; yeast extract, 1.5; beef extract, 1.5 and sodium chloride, 5; (pH 7 ± 0.2) and incubated at 37°C on rotary shaker at 180 rpm for 24 h. This was used as the inoculum for the keratinase enzyme production process. Submerged fermentation process was carried out by inoculating pure culture of potent isolate into the production medium (Suntomsuk and Suntomsuk, 2003). The production medium contains (g/L) Feather meal, 10; Yeast extract, 0.1; MgSO4, 0.1; NH4Cl, 0.5; K2HPO4, 0.3; KH2PO4, 0.3; NaCl, 0.5. Initial pH of the medium was adjusted to 7.2 ± 0.2 with Tris–HCl buffer. The medium was sterilized in an autoclave for 15 min at 121°C. The production medium was inoculated with 5% (v/v) of 24 h old inoculum culture of potent isolate containing approximately 2×106 cells/ml. The flasks were incubated on a rotary shaker at 37°C and 180 rpm for 4 days. The 10 ml fermented broth was centrifuged at 10,000 rpm for 20 min, and the supernatant was used as crude enzyme. The keratinase enzyme activity was assayed by taking 1.0 ml of crude enzyme properly diluted in Phosphate buffer (0.05 M of pH 7.0) was incubated with 1 ml of keratin solution at 50 °C in a water bath for 10 min, and the reaction was ceased by the addition 2.0 ml 0.4 M Trichloroacetic acid (TCA). The resulted precipitate was removed by centrifugation at 10000 rpm for 20 min. The absorbance of the supernatant was determined at 280 nm against a control. The control was prepared by incubating the enzyme solution with 2.0 ml TCA without the addition of keratin solution. One unit (U/ml) of keratinase enzyme activity was defined as an increase of corrected absorbance of 280 nm with the control for 0.01 per minute under the conditions described above.

RESULTS AND DISCUSSION

Table 1: Represent the total plate count of bacterial isolates for the obtained samples (commercial Feed, poultry litter and water).

S. No	CFU X Dilution factor
1	$3.9x10^{9}$
2	$3.5x10^{9}$
3	$5.5x10^{8}$
4	$2.5x10^{8}$
5	$3.2x10^{10}$
6	$1.5x10^{8}$
7	$3.5x10^{9}$
8	$4.9x10^{8}$
9	$3.2x10^{9}$
10	$3.8x10^{9}$

The goal of the current research was to identify microbes with the potential to keratin hydrolysis (keratinase activity) from poultry feed, litter, and water from poultry feeding operations. Using varied and selective culture media, 6 samples from each farm were evaluated. There were 5 different poultry farms involved. Here in the present study

Bacterial Isolates' Characterization: The collected bacterial isolates were subjected to various culture medium and biochemical characteristics (Table 2), which were chosen based on colony morphology, growth, and number of biochemical tests.

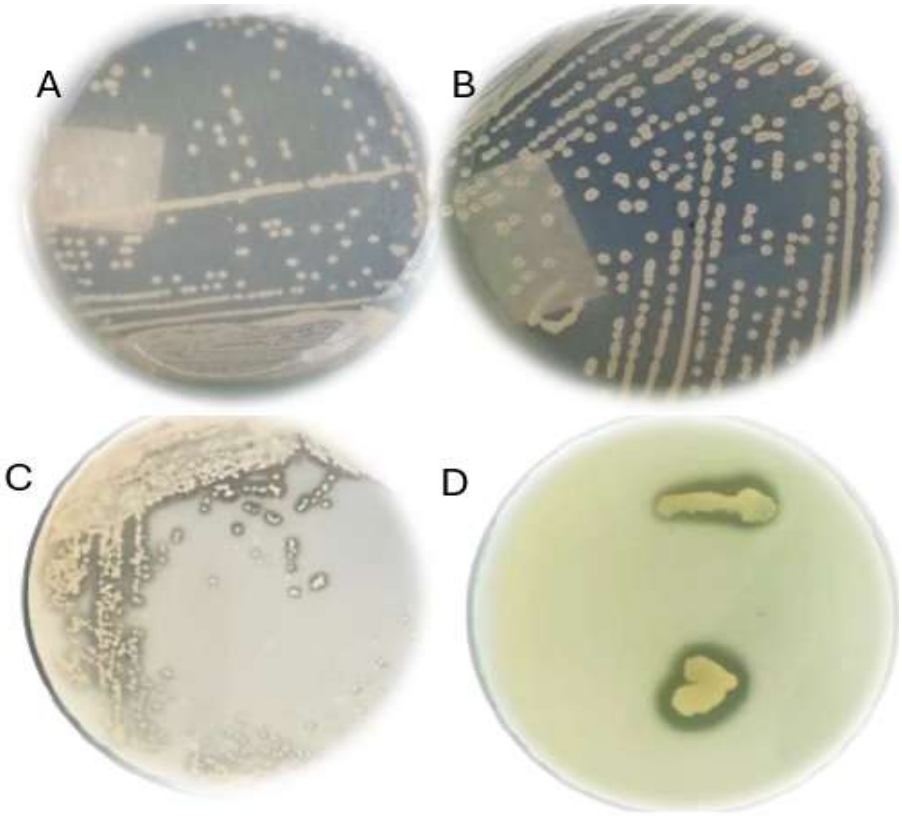

Figure 3: Isolated bacterial species from the poultry environment samples and setting. The key organism reported Bacillus licheniformis (A), Bacillus substilis (B), Shigella spp (C) and Proteus spp (D).

Table 2: Morphological identification of bacterial isolates

Morphological characterization of isolated bacteria	Gram Staining	Shape	organism
Green pigment-producing colonies	Gram –ve	Rod	*Pseudomonas* spp
Pink, convex with smooth-edged colonies	Gram –ve	Rod	*Escherichia coli*
Colonies with irregular margins, flat and white colour	Gram +ve	Rod	*Bacillus* spp
Colonies were white and swampy	Gram –ve	Rod	*Proteus* spp
Creamy, round, convex smooth Edged colonies	Gram –ve	Rod	*Salmonella spp,*
Shiny, spherical colonies on blood agar	Gram +ve	Cocci in chains	*Streptococcus* spp
Creamy and convex with Smooth edged colonies	Gram +ve	Cocci in clusters	*Staphylococcus* spp

ISSN: 0975-8585

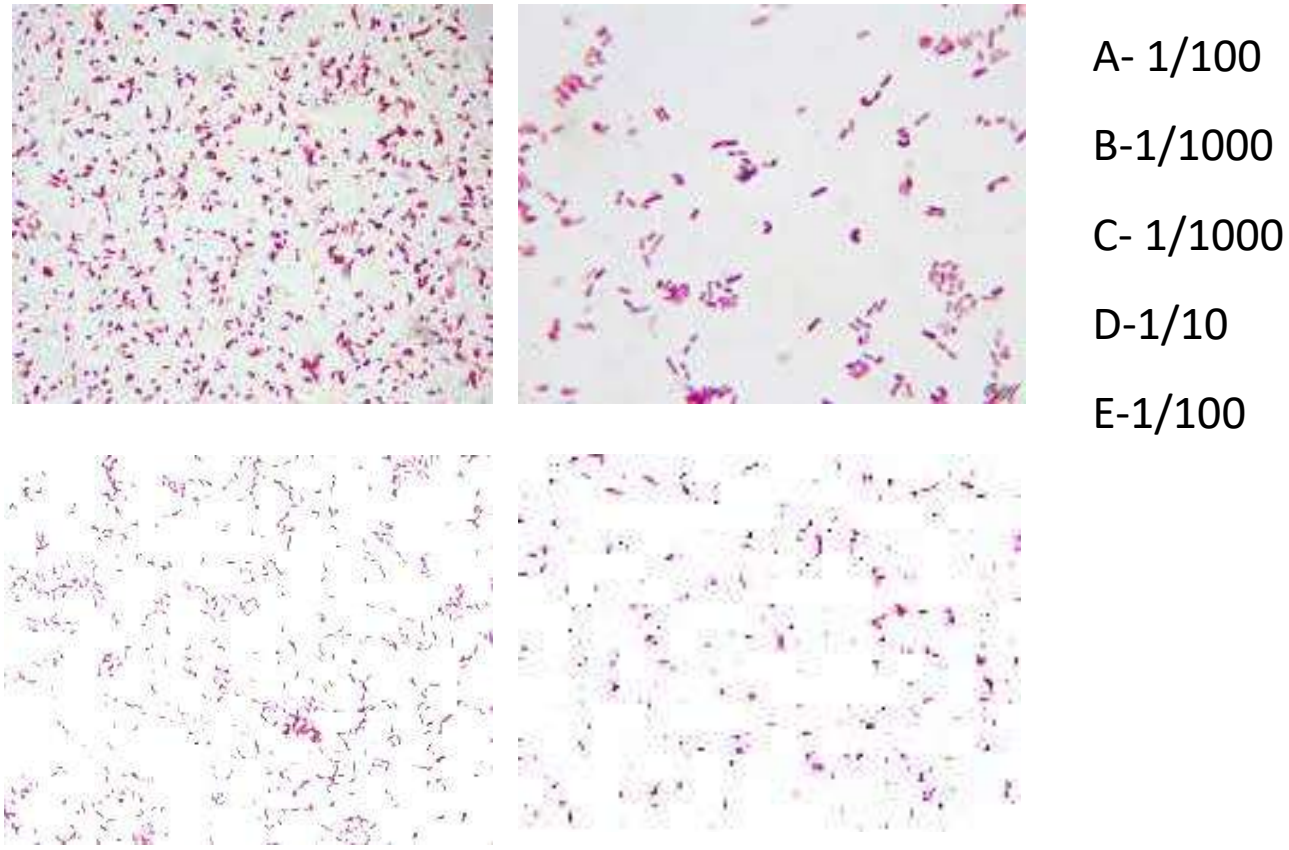

Figure 2: Gram staining and microsocpy of isolated bacterial culture isolated samples from poultery environmental settng.

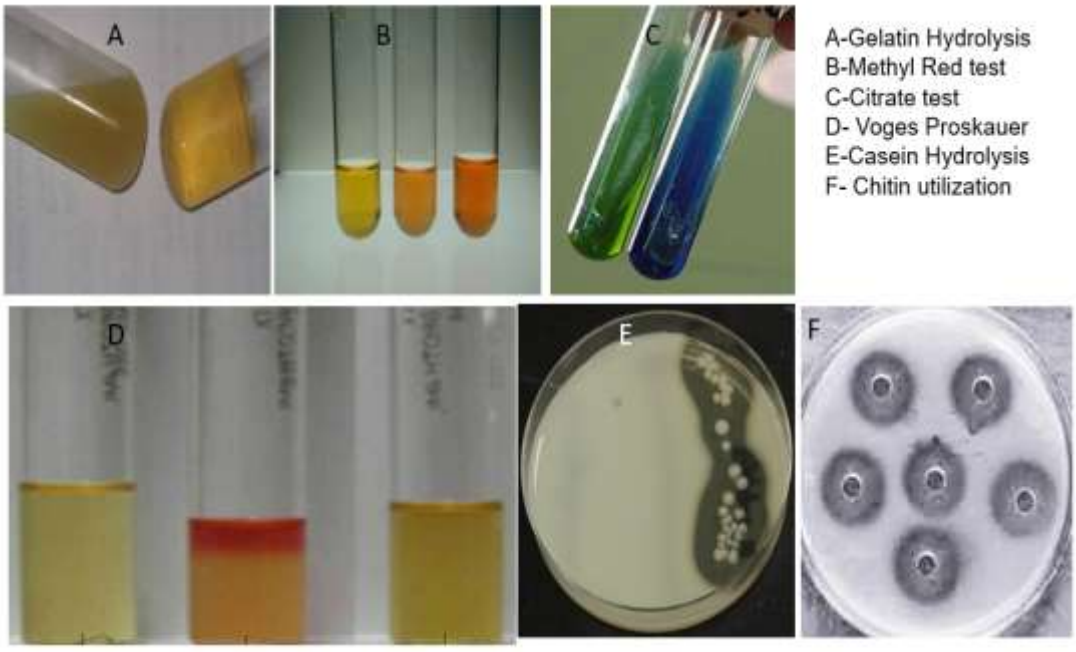

Figure 1: Biochemical analysis of isolated bacterial culture isolated samples from poultery environmental settng.

Table 3: Bacterial isolates distribution as sample wise poultry feed, poultry litter and water samples collected from the poultry setting.

S No	Sample	Bacterial species reported
1.	Poultry feed	Pseudomonas spp, E. coli, Salmonella spp, staphylococcus spp, Vibrio spp, Shigella spp
2.	Poultry litter	Salmonella spp, Pseudomanas spp, E. coli, Streptococcus spp, Bacillus substilis, Shigella spp
3.	Water sample	Pseudomonas spp, Shigella spp. Salmonella spp Vibrio spp

Table 4: Effect of inoculum size on enzyme activity.

S No	Incubation period (h)	Enzyme activity (U/ml)
1.	24	166 ± 2.10
2.	48	257 ± 2.14
3.	60	102 ± 11.6
4.	72	65 ± 1.3

CONCLUSION

The objective of the present study was to isolate the keratinase producing bacteria from chicken feathers, since it is rich in keratin. The production of enzyme keratinase and optimization of various parameters for the enzyme production were studied. This novel keratinolytic *Bacillus licheniformis* could be a potential candidate for the degradation of feather keratin and in dehairing process in leather industry. The enzyme activity of *Bacillus licheniformis* was reported 257 U/ml. This isolate could be used to produce keratinase for biotechnological applications and effectively used in the large-scale production of enzyme for commercial purposes.

ACKNOWLEDGEMENT

Author would like to thank Department of Microbiology, Chaitanya Deemed to be University, Hyderabad, India for providing facilities and support during the study.

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