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Isolation, Identification, And Characterization Of A Keratolytic Bacterium From Poultry Wastes

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Abstract: *The last two decades, feather pollution and feather waste management received many attentions, but unfortunately no solution was turned up strongly for feather waste management. Biological degradation of feather waste is more efficient than physical and chemical degradation, as its yielding more useful and toxic free by-product. From enrichment cultures, a total of 22 bacterial strains were obtained on medium containing feather as carbon source. All bacterial isolates are able of degrading milk protein on skimmed milk agar. Out of 22 isolates, the isolate AM9 showed the highest feather keratinase activity and hydrolyzed whole chicken feathers at 50°C within 7-10 days. Almost, all feather rachis was disappeared after 10 days. This isolate was identified as Bacillus amyloliquefaciens AM9. The activity of Keratinase and growth of the isolate AM9 was followed through cultivation in the basal broth containing feather as carbon and nitrogen source at 37-50°C and different initial pH values and different incubation periods. Keratinase production was the highest at 50°C (about 224 Uml^{-1}) after 24 h at pH 9. In conclusion, the hydrolysis of keratin wastes by bacteria is recommended for cleaning the environments.*

Keywords: *Feather, Degradation, Bacillus amyloliquefaciens, Keratinase*

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I. Introduction

Keratin-rich animal by-products such as chicken feathers, horns, and bristles are the third most plentiful renewable polymeric material current in nature after cellulose and chitin (1). Keratin waste biomass is classified as a low-risk animal by-product which occurs plentifully in meat & poultry processing plants. Therefore, keratin material is not suitable for human uses and its treatmentdecreased environment pollutions (2, 3). Keratin is mainly composed of proteins and mainly found in skin, nail, hair, horn, feather, and wool. High degree of cross-linking of disulfide bonds and hydrogen bonds are observed in Keratin fiber structures (4). The byproducts specially featherwastes are produced in large quantities during poultry processing and can be used for synthesis of pure materials like keratin, amino acids and soluble proteins (5). Although Keratin wastes are insoluble, extremely had rigid structures, and difficult to degrade,it can be degraded into feather meal and soluble protein for animal feeds. microbial or enzymatic treatment for hydrolysis of keratin to nutritional upgrading of feather meal has been described. Keratinase from bacteria is very important because this method is low energy consuming technology for changing of poultry feathers, potent pollutant,to important proteins, nutritionally upgraded fodders for man and animals. Bacteria secreted keratinase which degrade keratins. Keratinolytic activity has been reported for different bacterial and fungal genera (6). These enzymes enable keratinolytic bacteria and fungi to degrade waste keratin in nature (7). Keratinases have several potential applications including nutritional improvement of waste feather for live-stock feed, production of protein hydrolysate from keratinous waste materials (8), It is also true that knowledge of keratin hydrolysis mechanism is very restricted.

The study is dealing with a keratolytic bacterial isolated from the soil collected with feather waste in Jeddah, Saudi Arabia. The aim of present study is to isolate keratolytic bacteria from feather waste dumping soils, poultry farm soil samples in Jeddah, Saudi Arabia, followed by complete characterization of potential keratolytic bacterium with respect to identify it as well as study the factors affecting enzyme production.

II. Material And Method

Isolation of keratinolytic microorganism

Samples were collected from several sites (e.g., the dumping waste and soil from chicken farm) in Jeddah, Saudi Arabia. These samples were enriched in broth containing feather meal with compassion (g/l): NH_4Cl, (0.5); NaCl, (0.5); K_2HPO_4, (0.3); KH_2PO_4, (0.4); $MgCl_2{\cdot}6H_2O$, (0.1); feather powder, (10.0) (pH 7.5), in which feathers served as the sole source of carbon, nitrogen, sulfur and energy. The cultures were incubated at 37°C with stirring at 180 rpm for 7-10 days.

Screening for proteolytic activity
Test for proteolytic activity was carried out using skimmed milk agar as described by Harrigan and McCance (9).

Measurement of bacterial growth:
Bacterial growth was detected in the culture filtrate by measuring the optical density at 600 nm using spectrophotometer (Spectrophotometric, SHIMADZD). All experiments were carried out in triplicate and averages were calculated.

Bacterial growth in liquid medium:
Fifty ml of feather meal media (g/L); NaCl (0.5), KH_2PO_4 (0.7), K_2HPO_4 (1.4), $MgSO_4$ (0.1), and feathers (10), pH 7.2 were put in 250 ml flasks. After sterilization, the flasks were inoculated with 2 ml of bacterial precultures. All flasks were incubated for 7 days using shaking incubator (120 rpm and 37^{o}C). The cells were collected, and the filtrate was used to measure keratinase production.
Assays of enzyme activity
Keratin azure (Sigma-Aldrich, USA) was used as the substrate. Keratin azure powder was suspended in 1 ml 50 mmol/L Tris-HCl buffer (pH 8.0). The reaction mixture contained 1 ml keratin azure suspension and 1 ml appropriately diluted enzyme. The supernatant was spectrophotometrically measured for release of the azo dye at 595 nm. A control of 1 ml keratin azure suspension in the same buffer (like that of the sample) was agitated for 30 min at 50°C, then TCA was added and 1 ml enzyme solution.
One-unit (U) keratinase activity was defined as the amount of enzyme causing 0.01 absorbance increase between the sample and control at 595 nm under the conditions given.

Effect of different incubation temperature on enzyme production:
The flasks were incubated at different temperatures such as 25, 30, 37, 45 and 50 for 7 days with agitation at 200 rpm. At the end of the growth period, the growth and keratinase activity of the inoculated bacterium were measured as described before.

Effect of pH value of the medium on enzyme production:
After selection the best concentration of keratin, glucose and yeast extract, they were used for studying the effect of pH value on keratinase production of the selected bacterial isolate. The medium was prepared with different pH values (pH 5.0, 6.0, 7.0, 8.0 and 9). After preparation the different sterile media in 250 ml Erlenmeyer flasks containing 50 ml of the medium and inoculated with 2 ml of preculture of the selected bacterial, the flasks were incubated 7 days with agitation at 200 rpm at 37ºC. After growth period, the bacterial growth and keratinase activity were measured.

Effect of incubation period:
After selection the best pH of the medium, the effect of different incubation period (1, 3, 5, 7, and 10 days) was determined. After preparation the different sterile media in 250 ml Erlenmeyer flasks containing 50 ml of the medium and inoculated with 2 ml of the selected Bacterial preculture, the flasks were incubated at 37ºC with agitation at 200 rpm. After each growth period, the bacterial growth and keratinase activity were measured.

Statistical analysis:
Each reading had three replicates. Means of variable and standard deviation were recorded. Data were subjected to statistical analysis and difference between mean values determined by the Student's t-test. Differences were considered significant when probability was less than 0.05.

III. Result

A total of 22 bacterialisolate can grow in keratin medium and degrading skimmed milk. From all isolates, the isolate AM9 at 37°C was the most active in feather-degradation where itcompletely removed feather powderfor 7-10 days. All feather barbules and rachis were degraded after 10 days (Figure 1). Maximum growth in liquid medium was recorded for isolate AM9 (optical density at 600 nm measured using spectrophotometer was 1.4 while it was ranged from 0.64-1.11 for other isolates).

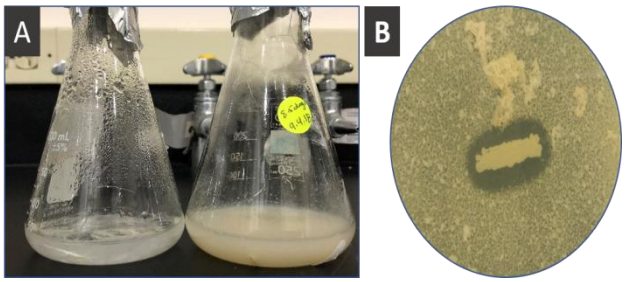

Figure 1: A) The flask on the left is control and the right flask is degraded whole father with bacterial isolate AM9 after 10 days. B) Clear zone on skimmed milk agar for isolate AM9.

Growth and Keratinase productionof the isolate AM9 was confirmed using mineral broth medium at initial pH 7 and containing one feather 50°C. The highestgrowth was at 30-37^{o}C while keratinase production was optimal at 50°C. The highest level of keratinase activity (about 226Uml -1) was produced at 24 h. An increase in the pH of the medium to above 11 was notable after 7 days of growth.

The isolate AM9 was characterized as rod-shape Gram-positive cells with yellowish color irregular rough colonies when grown on nutrient agar plates(Table1). The growth wasoptimal 30- 40°C while it decreased below 25°C or above 50°C. The growth was at pH 5.0 - 11.0 with an optimal at5-7. According to physiological and biochemical characters, the isolate AM9 was identified as*Bacillus amyloliquefacien*s(Figure 2).

Table 1: Morphological and BiochemicalCharacteristics of *Bacillus amyloliquefaciens*AM9

Characterization		Bacillus amyloliquefaciens AM9
Morphological	Appearance	Wrinkle, dull, dry
	Pigment	White
	Form	Irregular
	Margin	Lobate
Gram reaction		Gram positive
Endospore Fermentation		+
Catalase		+
Oxidase		+
Starch hydrolysis		+

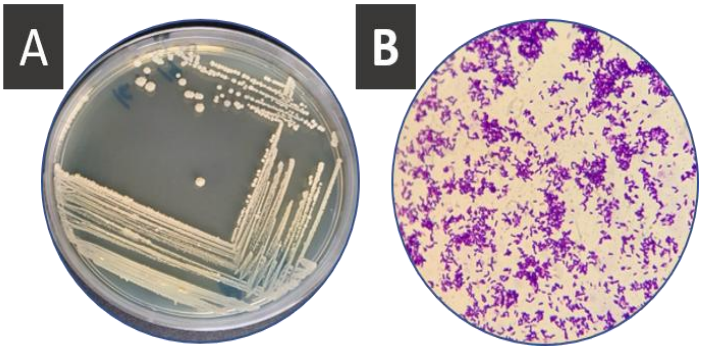

Figure 2: A) *Bacillus amyloliquefacien*s AM9 on Nutient Agar. B) *Bacillus amyloliquefacien*s AM9 under light microscope after Gram stain

Enzyme production by the tested bacterium was observed in the range of 25-50°C, with maximal activity at 50°C (Figure 3). The keratinase production by the tested bacterium was tested at different pH values. The keratinase production was obtained in a pH range of 3-11 with maximum activity at pH 9 (Figure 4).

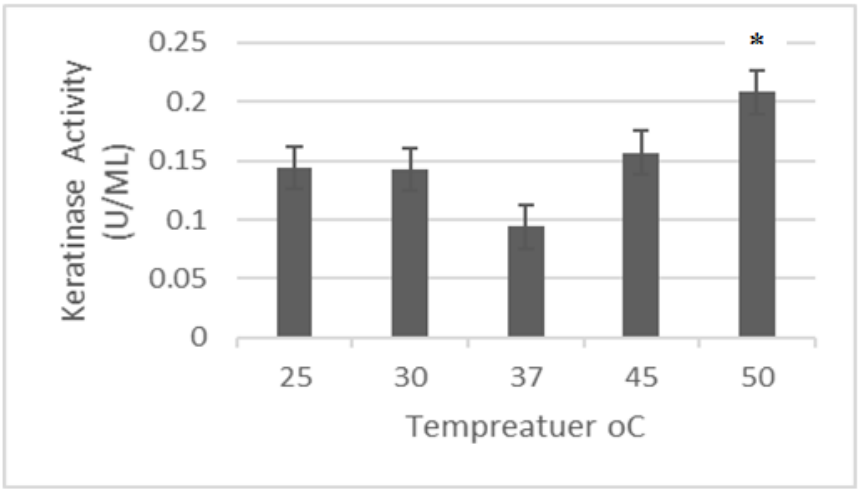

Figure 3: Effect of Temperature on keratinase production by the isolate AM9
*: significant results at P≤0.05 compared to control, grown at 37°C

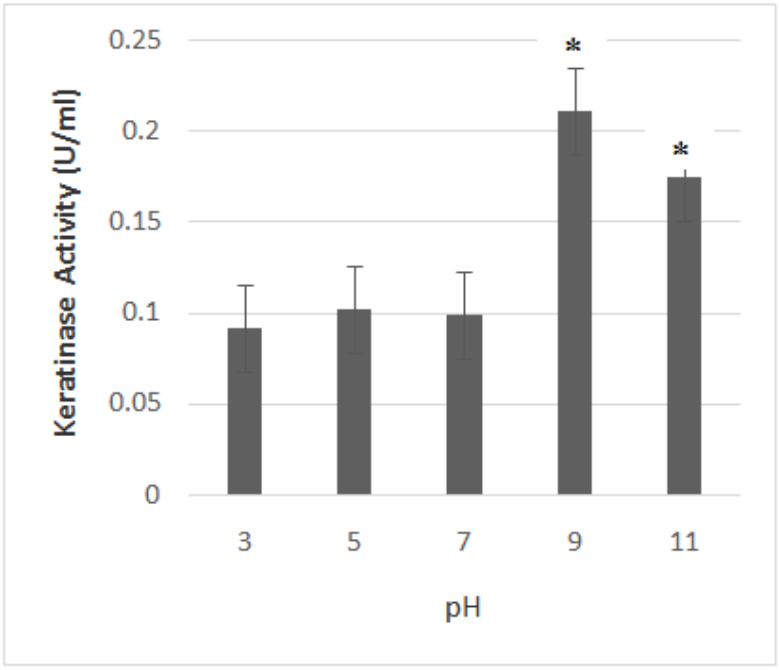

Figure 4: Effect of pH values on keratinase production by the isolate AM9
*: significant results at P≤0.05 compared to control, grown at pH7

IV. Discussion

Microorganisms and the keratinolytic enzymes that destroy feathers could be used to improve the digestibility of feather keratin (10, 11, 12) and their use as an animal-feed (13, 14). Morphological and physiological characteristics of the bacteria were compared with that of Bergey's Manual of Systemic Bacteriology. The identification of the isolate was confirmed as *Bacillus amyloliquefaciens. In* agreement with the results of this study, degradation of keratin Gram-positive bacteria, including *Bacillus, Streptomyces* and a few strains of Gram-negative bacteria, (15,16) has been reported. The isolation of keratinase producing strain of *Bacillus amyloliquefaciens* had been previously reported (17). In this study, *Bacillus amyloliquefaciens* demonstrated higher keratin degrading ability in whole intact feather samples. Contrary to this study however, other reports revealed that in bacteria, feather keratin-degrading abilities have been observed mostly in strains of *Bacillus amyloliquefaciens* (18).

In this study, the resulte of complete degradation of the feathers because of the incubation at the aerobic growth of all the isolates on feathers as the primary source of carbon, nitrogen, and energy. In agreement with the findings of this study, it has also been informed that in many strains, complete degradation of feathers was

achieved after 28 days (19). It was clear that enhancement of growth conditions increased the keratin degradation process (20).

V. Conclusion

The isolated *Bacillus amyloliquefaciens* AM9 on this study showed that could be benefit in the biotechnological management of poultry feathers through efficient biodegradation. Also, it can be improved the nutritional value of animal feeds that contain feathers (and other keratins).

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