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Isolation and Identification of Keratinolytic Bacteria that Exhibit Feather-degrading Potentials

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Authors' contributions

This work was carried out in collaboration between all authors. Author FOI designed the study and wrote the first draft of the manuscript. Author MYT performed the statistical analysis and wrote the protocol. Authors JO and BAD thoroughly read through scientific contents and made desired corrections in the final draft of the manuscript. Author JAI managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To isolate and identify feather degrading bacteria from soils collected from the feather dumping site.
Study Design: Isolation and preliminary identification of bacterial isolates with keratinolytic potentials.
Place and Duration of Study: Mudalawal poultry processing site, Bauchi state, Nigeria, between January 2014 to October, 2014.
Methods: Soil samples from feather dumping sites were screened for bacterial growth. The

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isolated bacteria were identified based on their morphological and biochemical characteristics and were tested for their ability to degrade whole intact feather and powdered feather samples.
Results: The results showed that three (3) of the isolates belonged to the genus *Bacillus* which include; *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus cereus*, while one isolate is a Gram-negative rod, *Serratia marcencens*. Although *B. licheniformis* demonstrated higher feather degrading ability but with no statistical difference from other isolates in degrading both powdered and intact whole feathers samples. Synergy among the isolates to degrade powdered feather showed no statistical difference (p=0.317). Also, synergy among all the isolates resulted in degradation of intact feather significantly higher than that of *S. marcescens*, but not significantly different from those of *B. licheniformis* (p=0.389), *B. subtilis* (p=0.096) and *B. cereus* (p=0.096). Moreover, the keratinolytic ability of the isolates on intact whole feather was time dependent. It was also observed that the rate of degradation of powder feather samples was significantly higher than the rate of degradation of intact whole feather by all the isolates (p=0.026).
Conclusion: These isolates are therefore promising organisms for the management of chicken feather waste through biotechnological processes.

Keywords: Bacillus; keratinolytic-bacteria; feather.

1. INTRODUCTION

Feathers are one of the epidermal growths that form the distinctive outer covering or plumage, on birds. Although feathers cover most part of the body of birds, they arise from certain well defined tracts on the skin. They aid in flight, thermal insulation, water proofing and coloration that help in communication and protection. Feathers are produced annually in huge amounts as waste products of commercial poultry-processing plants. Feathers represent 5 to 7% of the total weight of a mature chicken [1]. Feathers are produced annually in huge amounts as waste products of commercial poultry-processing plants. At present these are either buried in landfills or incinerated in a power plant generator boiler. Although land application is an option, continued application can result in extreme high soil nitrogen levels with run-off contaminating streams and ground water with both chemicals and bacteria. A current value-added use for feather is its conversion, following treatment at high temperature and milling, to feather meal/ animal flour and used as a protein supplement into feed mixtures of domestic animals. However, production of feather meal is an expensive process which destroys certain amino acids, yielding a product with poor digestibility and variable nutritional quality [1,2]. Dynamic hydrolysis by microorganisms that possess keratinolytic activity represents an attractive alternative to improve the nutritional value of feather wastes.

Feathers are composed mainly of keratin. Keratins are insoluble fibrous proteins found not only in feathers but also in hair, wool [3], nail, horns [4] and other epithelial coverings [5]. Keratin is hard to degrade due to highly rigid structure rendered by extensive disulphide bond and cross-linkages. The keratin chain is insoluble, high stable structure tightly packed in the "*α*-helix ("*α*-keratin) and *β*-sheets (*β* -keratin) into super coiled polypeptide chain [6]. 90% of the feather contains *β*-keratin by mass [7] and *β*-keratin is extensively cross linked. Keratinolytic microorganisms and their enzymes may be used to enhance the digestibility of feather keratin with important applications in processing keratin-containing wastes from the poultry industry and the nutritional upgrading of feather meal which could replace as much as 7% of the dietary protein for growing chicks [8].

At present, the keratinolytic activity has been reported for *Bacillus* species [9,10, 11,12], *Fervidobacterium* sp. [13,14], *Thermoanaerobacter* sp. [15], *Xanthomonas* sp. [16], *Vibrio* sp. [17], *Microbacterium* sp. [18] and *Candida parapsilosis* [19], as well as fungi [20, 21,22], *Actinomycetes* [23,24,25]. In this study, we described the preliminary isolation of bacteria from poultry processing industry showing keratinolytic activity.

2. MATERIALS AND METHODS

2.1 Sample Collections

The soil sample containing degraded feather was collected from the feather dumping site located in Mudalawan market poultry processing industry. All the samples were transported in a plastic bag to the laboratory for further microbiological analyses.

2.2 Isolation of Feather Degrading Bacteria

A ten-fold Serial dilution from each sample was prepared by adding 1 g of the soil sample to the 9 ml of sterile distilled water. The diluted water sample plated on Nutrient Agar medium and incubated at 35ºC for 24 hours. The appeared colonies were checked for spore presence and streaked on agar slant for further characterization [26].

2.3 Identification of Bacterial Isolates

Isolates were identified based on cultural, morphological and biochemical characteristics and were compared with the [27].

2.4 Degradation of Feather by the Isolates under Laboratory Conditions

2.4.1 Degradation of intact whole feather

Feathers that were collected were thoroughly washed with tap water, rinsed 3 times with distilled water, dried overnight at room temperature and weighed to the nearest milligram. One gram of the feather was placed in 8 ml of nutrient broth and autoclaved 15 min at 120ºC [17]. Each isolate was inoculated in 4 replicate tubes. Another set of four tubes were inoculated with all the isolates. Inoculations were adjusted to a final concentration of 1.6 x 10^6 cells per ml. Control tubes were not inoculated. The tubes were incubated for 4 weeks at 37ºC. Residual feathers from each experiment were harvested weekly for 4 weeks. The harvested residual feathers were dried to a constant weight in hot air oven at 50ºC. Remaining feathers and fragments collected on the membrane filter were reported as the percentage of weight compared to the initial dry weight of the feather.

2.4.2 Degradation of powdered feather in nutrient broth

The method described by Lucas et al. [28] but slightly modified was applied in this study to show Keratin solubilization of milled feathers. Feathers from the poultry processing plant were washed, dried as described above, cut in small pieces, and reduced in a coarse powder using a mortar. A quantity of 1.0 mg of feather powder was resuspended in 5 ml of nutrient broth and autoclaved as above. For each isolates, three tubes were inoculated with 0.2 ml of bacterial suspension of about 1.2 x 10^6 cells per ml. Another set of three tubes were inoculated each with all the isolates and the tubes were all incubated for seven days at 37ºC. Control tubes were not inoculated. Residual feathers were harvested from the fermentation broth by filtering it over whatman filter paper No. 2 and were kept in hot air oven at 50ºC until constant weight was obtain. The difference between the weight of residual feather obtained and that of initial weight before incubation was used as measure of feather degradation.

2.5 Statistical Analyses

Anova and Mann-Whitneys test were used to test for significance difference in all the data obtained. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non- significance difference was defined when $p \leq 0.05$ and $p > 0.05$ respectively.

3. RESULTS

Based on cultural and biochemical characteristics of the isolates as shown in Table 1, a total of four feather degrading bacteria were successfully isolated from poultry processing waste dumps in Bauchi. These bacterial species are; *Bacillus licheniformis, Bacillus subtilis, Bacillus cereus* and *Serratia marcescens*. The result in Table 2 showed the degradation of powdered feather by the isolates. The results revealed that *B. licheniformis* demonstrated a high feather-degrading activity by degrading *0.6g* (60%) of the powdered feather sample, while the least was shown by *S. marcescens*, 0.3 g (30%). The result further showed that synergy among the four isolates increases feather degrading ability up to 80%. However, degradation of the powdered feather by the individual isolates did not differ significantly from each other and also did not differ significantly from all the isolates combined together (p=0.317). Degradation of whole intact feather by the isolates weekly for four weeks was represented in Table 3. None of the isolates was able to degrade the intact feather after one week. However, synergy among the isolates resulted in the degradation of only 0.1 g (10%) of the intact feather after the first week. The degradation of the intact feather after the 2nd and 3rd week was similar among all the *Bacillus* spp, but *Serratia marcescens* wasn't able to degrade intact feather after the 2nd and 3rd week. At the end of the fourth week, *B. licheniformis* showed the highest degrading

capability by degrading 0.7 g (70%) of the whole intact feather. Synergy among the isolates at the end of the fourth week resulted in the degradation of feather equal to that of *B. licheniformis*. However, the amount of intact whole feather degraded by *Bacillus licheniformis* differ not significantly from those degraded by *Bacillus subtilis (p=0.389), Bacillus cereus (p=0.389)* and *Serratia marcescens* (*p=0.096).* The results further showed that synergy among all the isolates resulted in degradation of intact feather significantly higher than that of *S. marcescens*, but not significantly different from those of *B. licheniformis* (p=0.389), *B. Subtilis* (p=0.096) and *B. cereus* (p=0.096). The results further showed that the rate of degradation by all the isolates in week one differ not significantly to the rate of degradation in week 2 and week 3 (p=0.073). However, degradation of the intact feather by all the isolates at the end of week 4 was significantly higher than that of week 1 (p=0.002) and week 2 (p=0.014), but not significantly different from the rate of degradation in week 3 (p=0.118). Also, the rate of degradation of powder feather samples was significantly higher than the rate of degradation of intact whole feather by all the isolates (p=0.026).

4. DISCUSSION

Microorganisms that degrade feathers and their keratinolytic enzymes could be used to enhance the digestibility of feather keratin [29,30,31] in the degradation of feathers and their utilization as a feedstuff [17,32].

All isolates showed good growth with distinct characteristics on nutrient agar. Morphological and physiological characteristics of the bacteria were compared with the Bergey's Manual of Systemic Bacteriology. Three of the isolates were Gram-positive, rod shaped and spore-former, while the fourth isolate was a Gram-negative bacterium. The organisms isolated were confirmed to be *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus cereus* and *Serratia marcescens*. In agreement with this study, degradation of keratin has been reported to be mostly confined to Gram-positive bacteria, including *Bacillus*, *Streptomyces* and a few strains of Gram-negative bacteria, [33,34]. The isolation of keratinase producing strains of *B. subtilis, B. licheniformis and B. cereus* had been previously reported [35,36,37,38,39,40]. Many *Bacillus* species have been reported to possess keratinolytic activities [41,42,43,44, 29,30,31]. However, Streptomyces sp. were not isolated in this study, which may be due to the fact that Streptomycetes are slow growing and were probably overgrown by other strains. This is similar to another finding previously reported [28].

In this study, *Bacillus licheniformis* demostrated higher keratin degrading ability in both powdered feather sample and whole intact feather samples with no significant different from other isolates. This implies that all the isolates have equal keratin degrading ability. Contrary to this study however, other reports revealed that in bacteria, feather keratin-degrading abilities have been observed mostly in strains of *Bacillus licheniformis* [45,43], less frequently in populations of *Bacillus pumilis, Bacillus cereus and Bacillus subtitis* [38].

The keratinolytic activity of all isolates increased with the increase of time as shown in this study. This is in agreement with previous report [1].

In our study, the aerobic growth of all the isolates on feathers as the primary source of carbon, nitrogen, energy and sulphur, resulted in partial degradation of the feathers after 7days of incubation (for powdered feather) and 28 days (for whole intact feather); the barbules and rachises were partially degraded by both strains. In agreement with the findings of this study, it has also been reported that in many strains, complete degradation of feathers was not achieved, as the rachis was not fully degraded [46]. Contrary to this study however, complete degradation of feather including its barbules and rachises after 6-9 days of incubation were previously reported [35,1]. There are reports of complete or partial degradation of chicken feathers by bacteria in the range of 4-10 days [35,47,29,44,48].

The results of this study also showed that the rate of degradation of powder feather samples was significantly higher than the rate of degradation of intact whole feather by all the isolates (p=0.026). Higher keratinolytic activity of the isolates on the powdered feather samples might be due to the fact that grounding the feather into powdery form resulted in the increase of its surface area comparable to the whole intact feather.

Table 1. Cultural and biochemical characteristics of the isolates

Colonial/cell morpology	Biochemical Tests													Probable organisms
	Gram stain	Motility	Catalase	Coagulase	Indole	Cit utilization	Vog prosk	Methyl red	H. sulphide	Glucose	Lactose	Sucrose	Manitol	
Colonies are cream in appearance, circular form	+	+	+	-	+	+	+	-	-	-	A	A	AG	*Bacillus licheniformis*
Colonies are large not as large as *B. cereus* margin is undulated, with circular form and flat elevation.	+	+	+	-	+	+	+	-	-	-	-	-	-	*Bacillus subtilis*
Colonies are white in appearance, large, irregular and flat with undulated margin.	+	+	+	-	-	+	+	-	-	A	-	-	-	*Bacillus cereus*
Colonies are red in appearance, circular and irregular margins	-	+	+	-	-	+	+	-	-	AG	-	-	-	*Serratia marcescens*

Key + = Positive, - = Negative, A= Acid production. AG = Acid and gas production

Table 2. Degradation of powdered feather by the isolates

Isolates	Initial weight (g)	Final weight (g)	Amount degraded (g)	% degraded
Bacillus licheniformis	1.0	0.4	0.6	60
Bacillus subtilis	1.0	0.6	0.4	40
Bacillus cereus	1.0	0.6	0.4	40
Serratia marcencens	1.0	0.7	0.3	30
All the isolates	1.0	0.2	0.8	80

Table 3. Degradation of intact whole feather by the isolates

Isolates	Degraded time (week)	Initial wt	Final wt	Amount degraded	% degraded
Bacillus licheniformis	1	10.	1.0	0	0
	2	1.0	0.9	0.1	10
	3	1.0	0.7	0.3	30
	4	1.0	0.3	0.7	70
Bacillus subtilis	1	1.0	1.0	0	0
	2	1.0	0.9	0.1	10
	3	1.0	0.8	0.2	20
	4	1.0	0.7	0.3	30
Bacillis cereus	1	1.0	1.0	0	0
	2	1.0	0.9	0.1	10
	3	1.0	0.8	0.2	20
	4	1.0	0.7	0.3	30
Serratia marcescens	1	1.0	1.0	0	0
	2	1.0	1.0	0	0
	3	1.0	1.0	0	0
	4	1.0	0.9	0.1	10
All the isolates	1	1.0	0.9	0.1	10
	2	1.0	0.7	0.3	30
	3	1.0	0.5	0.5	50
	4	1.0	0.3	0.7	70

Considering that feather protein has been shown to be an excellent source of metabolizable protein [49], and that microbial keratinases enhance the digestibility of feather keratin [50,8], these keratinolytic strains could be used to produce animal feed protein in addition to the biodegradation of poultry wastes, including composting [51]. In fact, large-scale composting of keratin wastes, especially poultry feathers that are produced in the greatest amounts, may provide a solution to the problem of their utilization. It will also prevent wasting of the material and the ecological hazards resulting from keeping those wastes on waste dumps. Currently, over 30% of the total Industrial enzyme market is accounted by proteases used for detergent, leather tanning and food production. Use of feather, a cheap and readily available substrate, could result in a substantial reduction in the lost of enzyme production.

5. CONCLUSION

Although complete degradation was not achieved by all the isolates used in this study, these results showed that these bacteria could be useful in the biotechnological management of poultry feathers through efficient biodegradation. Also, the isolates can be expected to improve the nutritional value of animal feeds that contain feathers (and other keratins)

LIMITATIONS OF THE STUDY

Soil sample used in this study were exclusively taken from the natural composting site in the poultry processing industry and organisms isolated from the samples were only identified based on morphological and biochemical characteristics then compared to Bergey's Manual of Determinative Bacteriology.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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