



Chicken feathers degrading bacteria isolated from flamingo feathers in Lake Nakuru, Kenya

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Abstract

This study investigated the ability to degrade chicken feathers by bacteria isolated from flamingo feathers in Lake Nakuru which can be applied in the degradation of poultry and abattoir wastes for the production of protein supplement for animal feed formulations. Proteolytic activity of the isolates was screened on casein agar and their efficiency ranged from 3 to 27 mm. Two bacteria isolates; LNC06 later identified as *Bacillus agaradhaerens* by 16S rDNA sequencing and LNN03 which were found to have high potential in feather degradation. They were grown on chicken feather substrate and growth of bacteria was evidenced by increase in turbidity which was measured using optical density (DO) method and by reduction in dry weight and ash free dry weight. Their degradation performance in relation to temperature and pH was also measured using DO and the optimum growth in feather hydrolysis was achieved at a temperature of 35 °C and pH of 10. Reduction in feather dry weight and ash free dry weights were both showing significant correlations ($p < 0.05$) for the test bacteria. Results showed that both bacteria were good feather degraders but *Bacillus agaradhaerens* exhibited remarkably higher abilities than LNN03. Evidence from this study indicated that *Bacillus agaradhaerens* has high potential for application in feather degradation but further studies should be carried out to optimize the production and investigate the products for suitability as animal feeds protein supplements to improve the health of farm animals.

Keywords: *Bacillus*, Chicken feathers, Keratin, Degradation, Animal feeds supplements

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1. Introduction

Chicken feather wastes are produced globally in high amounts from poultry farms and restaurants in towns and cities. Their disposal is a challenge because of the keratin which is an insoluble protein with cross-linking disulphide bonds that makes it difficult to degrade by normal protease enzymes. Bo Xu *et al.* (2009) has been able to degrade chicken feathers through thermal degradation in high energy consumption industries a method that is not sustainable nor environmentally friendly. Keratin in feathers is insoluble to most common protease enzyme such as papain, trypsin and pepsin (Matikevičienė *et al.*, 2009).

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Bacteria with keratinolytic enzymes have been isolated from feather waste in landfills, non-treated soils in natural compost and poultry feather wastes (Joshi et al., 2007; Onuoha and Chukwura, 2011). Unfortunately, their degradative ability has been slower and unsteady causing massive pile ups of chicken feathers in landfills and pits. The most common disposal method being applied to rid of these wastes from our environment is combustion which unfortunately produces toxic fumes and increases greenhouse gases. Microbial conversion of feathers is a safer and more reliable method but depends on efficient microbes which are rarely present in landfills.

Lake Nakuru is an alkaline saline lake found in the Rift Valley in Kenya. It has a high number of lesser flamingos with up to 500,000 birds recorded at one particular time (Owino et al., 2001). Although flamingos are usually in high numbers, their presence has never resulted in feather wastes menace in the lake. Bacteria in this extreme environment have unique and high degradative ability that manages the feather wastes. *Bacillus* have been found to possess keratinolytic enzymes that could be utilized in hydrolyzing chicken feathers making the amino acids in feather leachate available with potential as feed supplements to domestic animals. These enzymes may be useful in improving the digestibility of keratin in feathers (Sekar et al., 2015). The alkaline saline environment characterized by high pH stabilizes proteolytic enzymes in bacteria increasing their catalytic properties as compared to those found in chicken feather bacteria from landfills.

Bacteria with protease enzyme were sought from flamingo feathers in the lake. These bacteria were then tested on chicken feathers for hydrolytic activities. No research has been done on feathers degrading bacteria from flamingo feathers and therefore this formed an important scientific opportunity to conduct research in this area.

The results from this study have helped enhance our knowledge on the potential use of such microbes in the production of animal protein feeds supplements from feathers.

2. Materials and methods

2.1. Sample collection and isolation of bacteria

Degrading flamingo feathers were collected from the shores of Lake Nakuru aseptically by use of sterilized pair of forceps and placed in sterile sampling bags for isolation of feather degrading bacteria. The flamingo feathers were incubated in 5 g^l⁻¹ peptone solution at 34 °C for 24 h. The bacterial suspensions were then streaked on nutrient agar, 4% NaCl, and 1% (w/v) Na₂CO₃ (for pH adjustment) for isolation of alkaliphilic bacteria from soda lakes as per the modified formulation of Horikoshi, (1971) and Grant, (2006). Pure cultures of bacteria were made by streaking the isolated colonies on sterile Trypticase Soy Agar (TSA) which was made with saline lake water and pH poised by the addition of 1% (w/v) of Na₂CO₃ in the TSA (Grant, 2006) and incubated at 30 °C for 24 h (Grant, 2006). The isolates were streaked several times on TSA to obtain pure cultures. Pure cultures were then stored on agar slants in the refrigerator at 4 °C for use in subsequent experiments.

2.2. Characterization of isolates

Out of the six proteolytic isolates from Lake Nakuru (results from the study by Musikoyo et al. (2015)), two bacterial isolates that exhibited high proteolytic degradation potential were morphologically and biochemically characterized according to procedure described in Bergy's Manual of Systematic bacteriology (Whitman et al., 2009). Isolate LNC06 was molecular characterized and identified as *Bacillus agaradhaerens* (details of the procedures are as described in (Musikoyo et al., 2015)). Classification of the two isolates as Gram positive or Gram negative was done by Gram stain reaction and KOH sensitivity test (Gregerssen, 1978). For KOH sensitivity test a heavy mass of 24 h bacterial and the cells suspensions on a slide was mixed rapidly with circular motion with an inoculation loop for 15-30 sec. The formation of a string (DNA) in 3% (w/v) KOH indicated that the isolate is a gram-negative organism. *Escherichia coli* and *Bacillus megaterium* were used as gram negative and gram-positive controls, respectively. Cultural characteristics of bacteria such as the color of the colony, the texture, shape and measurement of colony diameter were determined. Endospore presence was determined using a wire loop to smear a colony of bacteria on a microscope slide and distilled water was used to spread the colony. A drop of malachite green was added and the slide was placed over a beaker containing boiling water. The wet stain condition was maintained for 10 min. The slide was then removed from the water bath and allowed to cool to room temperature. Once the slide was cooled it was rinsed with tap water to remove the malachite green and was then counterstained with a drop of basic fuchsin (Beishir, 1991). After a minute the slide was rinsed and dried with bibulous paper and examined on a microscope under oil immersion objective. Endospores appeared as green bodies within the cells.

2.3. Screening for feather degradation

Two of the isolated bacteria LNN03 and LNC06 (identified as *Bacillus agaradhaerens*, procedure in (Musikoyo et al., 2015)) were compared for degradation of chicken feathers. Healthy and clean chicken feathers were selected from poultry holding in Ngongongeri Farm at Egerton University in Nakuru County, Kenya. The feathers were cut into pieces of 2 cm and thoroughly cleaned and placed in Erlenmeyer flask (250 ml volume) covered with an aluminum foil and then sterilized in the autoclave at 121 °C for 15 min. The sterilized feather suspension weighed at 4 g was used as the sole carbon source in the experiment. The content was incubated with the test bacteria in a flask at optimum conditions of pH, salinity and temperature for growth (conditions similar to that of the lake water). The test bacteria inoculum was prepared by picking a loop from the cultures and suspending in saline water to have an OD_{600nm} of about 0.4 containing 10⁶ bacteria cells per ml (Zerdani et al., 2004). A 5 ml aliquot from the content was taken and the optical density measured at 600 nm at given time intervals. The dry weight of feather was determined by collecting, a piece of feather from the feather suspension and placing it in pre-weighed crucibles and then dried at 60 °C for 24 h after which they were ashed at 500 °C for 4 h to obtain the ash free dry mass. This was done after every 24 h to the inoculated feathers. The dry weight and ash free dry weights were recorded.

2.4. Effect of temperature and pH on the growth of *Bacillus agaradhaerens* (LNC06) and LNN03

Broth medium (100 ml) with 1 g clean chicken feather and containing inorganic nutrients 1.0 g/l K₂HPO₄, 0.1 g/l MgSO₄·7H₂O, 1 g/l yeast extract and 0.05 g/l CaCl₂·7H₂O (Williams et al., 1990) was dispensed into each 3 triplicate 250 ml Erlenmeyer flasks and sterilized at 121°C for 10 min. The triplicates were inoculated with the two test bacteria separately and incubated at 25, 30, 35, and 40 °C for 10 days. Feather degradation by *Bacillus agaradhaerens* (LNC06) and LNN03 were monitored after 24 h by withdrawing about 5 mls aliquot and measuring the optical density (OD_{600nm}).

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3. Results

3.1. Bacterial isolates from flamingo feathers

The colonies of the two isolates were white/cream in color, opaque and rough in texture (Table 1).

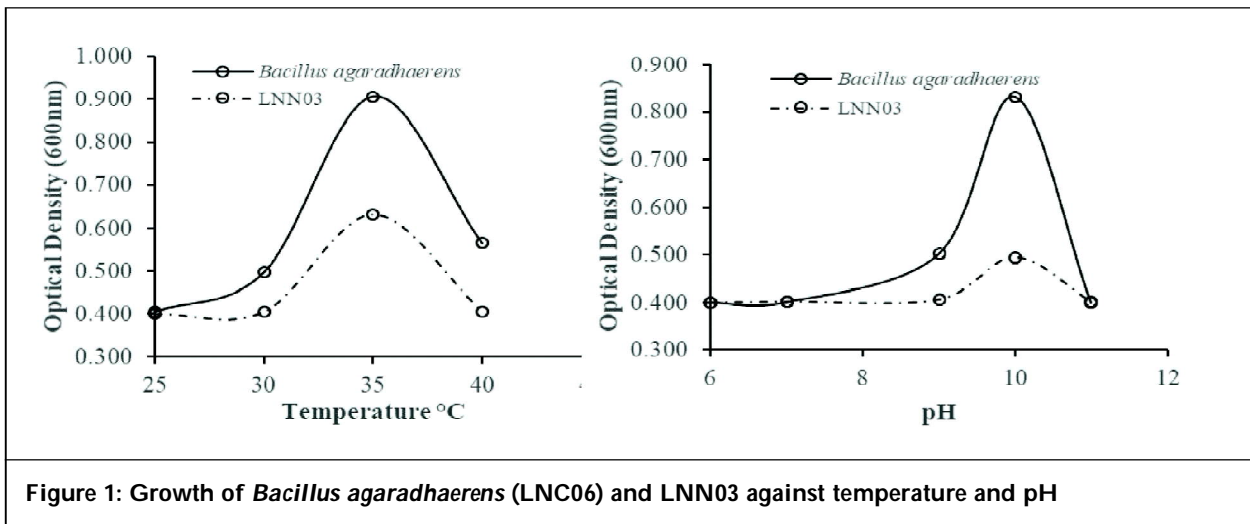
Characteristics	Isolates	
	LNN03	LNC06
Form	circular	irregular
Size (mm)	3.0	27.0
Edge	entire	undulate
Optical Characteristics	opaque	opaque
Texture	rough	rough
Color	cream white	white
Gram Reaction	-	+
3% KOH	+	-
Shapes	cocci	rods
Endospores	absent	present

Bacillus agaradhaerens (LNC06) and isolate LNN03 were tested on clean chicken feathers as the sole carbon source. The two isolates were capable of degrading chicken feathers with *Bacillus agaradhaerens* (LNC06) degrading feathers faster and completely than LNN03 (Plate 2).



3.2. Growth of *Bacillus agaradhaerens* and LNN03 at different temperatures and pH

The growth of bacteria was recorded at temperatures above 20 °C. Optimum temperature for *Bacillus agaradhaerens* was 35 °C. As the temperature increased to 40 °C the growth reduced as shown by the fall in optical density values (Figure 1). Isolates LNN03 exhibited similar pattern in terms of temperature effect on its growth but was slower as compared to *Bacillus agaradhaerens*. It grew at temperatures above 20 °C with an optimum temperature of 35 °C. The lowest recorded growth for the two isolates was at pH 6, as indicated by the low optical density of 0.4 (Figure 1). Their growth was not well differentiated at pH <7, but they exhibited optimal growth at pH 9–10. *Bacillus agaradhaerens* grew faster than LNN03 at pH 10. pH values >10 decelerated growth as shown by the fall in optical densities.



3.3. Degradation of chicken feathers by *Bacillus agaradhaerens* (LNC06) and LNN03

The results showed that *Bacillus agaradhaerens* (LNC06) had a growth pattern superior than that of LNN03. There was no noticeable difference in optical density between isolate LNN03 and *Bacillus agaradhaerens* (LNC06) after 24 h of incubation. The mean optical density value of isolate LNN03 was 0.401 with an increase in degradation being observed as from day 3 to day 10. The optical densities of the isolates increased notably from day 2 with *Bacillus agaradhaerens* (LNC06) exhibiting remarkable growth as from day 4 to day 10 (Figure 2).

As expected, the mean dry weight of feathers decreased with increase in bacteria growth in the feather medium. The feather meal inoculated with *Bacillus agaradhaerens* (LNC06) showed the highest decrease in

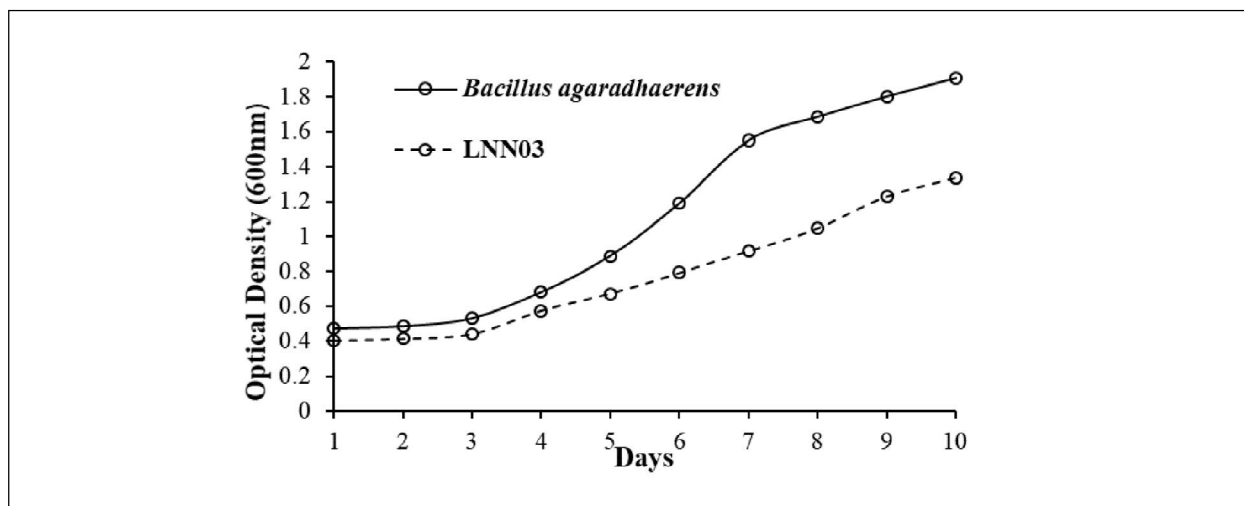


Figure 2: Growth pattern of *Bacillus agaradhaerens* (LNC06) and LNN03 on chicken feather

weight during the period of experiment. At the beginning of the experiment, LNN03 degraded the feathers faster from 0.107 g to 0.059 g in day 4 (Figure 3). *Bacillus agaradhaerens* (LNC06) picked up the degeneration pace from day 4 to day 10 (0.069 g to 0.013 g). Ultimately, *Bacillus agaradhaerens* (LNC06) performed better in the experiment.

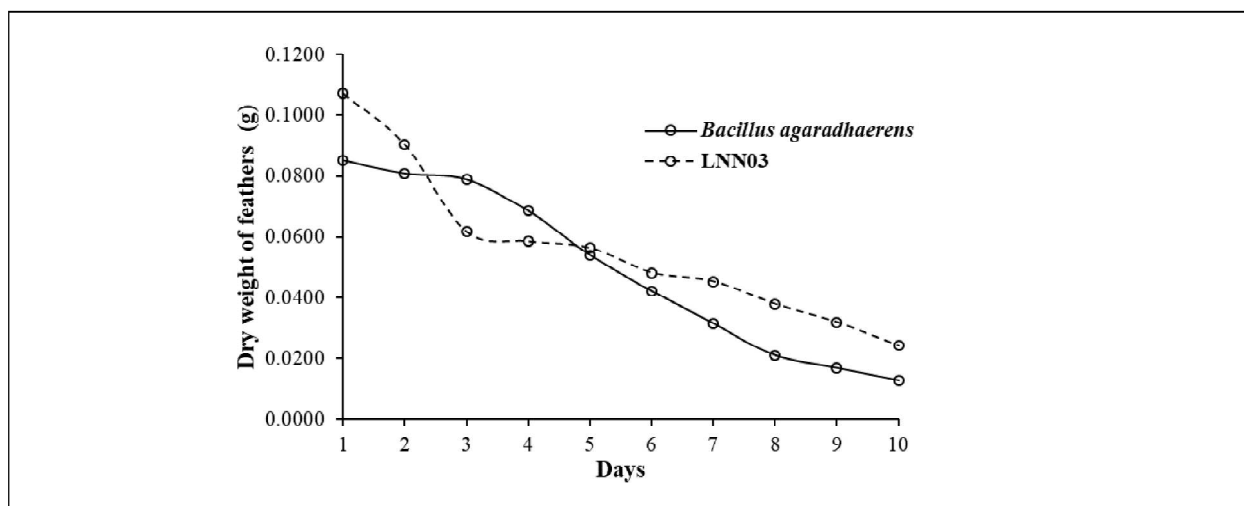


Figure 3: Profiles showing changes in dry weight of chicken feathers during the assay inoculated with isolates LNC06 and LNN03 from Lake Nakuru for 10 days

The ash free dry weight of the feathers decreased with time during incubation. *Bacillus agaradhaerens* (LNC06) devoured the feathers from 0.022g to 0.0006g in 10 days. Despite both organisms degrading the feather substrate, LNN03 was slower.

There was a statistically significant difference in optical density of *Bacillus agaradhaerens* (LNC06) and LNN03 (Independent t -test $t = 2.844$, $df = 58$, $p < 0.05$). Conversely, there was no statistically significant difference in dry weight and ash between *Bacillus agaradhaerens* (LNC06) and LNN03 (Independent t -test $t = -1.038$, $df = 58$, $p > 0.05$ and Independent t -test $t = -1.242$, $df = 58$, $p > 0.05$ respectively).

Optical density had an inverse relationship with the dry weight of feathers as shown in Figure 5 ($R^2 = 0.8335$). Pearson correlation analysis confirmed a significant correlation between optical density and dry weight of chicken feathers ($R^2 = 0.913$, $p < 0.01$, $n = 60$).

There was a positive correlation between dry weight and ash free dry weight of the feathers ($R^2 = 0.8233$) Figure 6. Pearson correlation analysis also indicated significant positive correlation between the two variables ($R^2 = 0.907$, $p < 0.01$, $n = 60$).

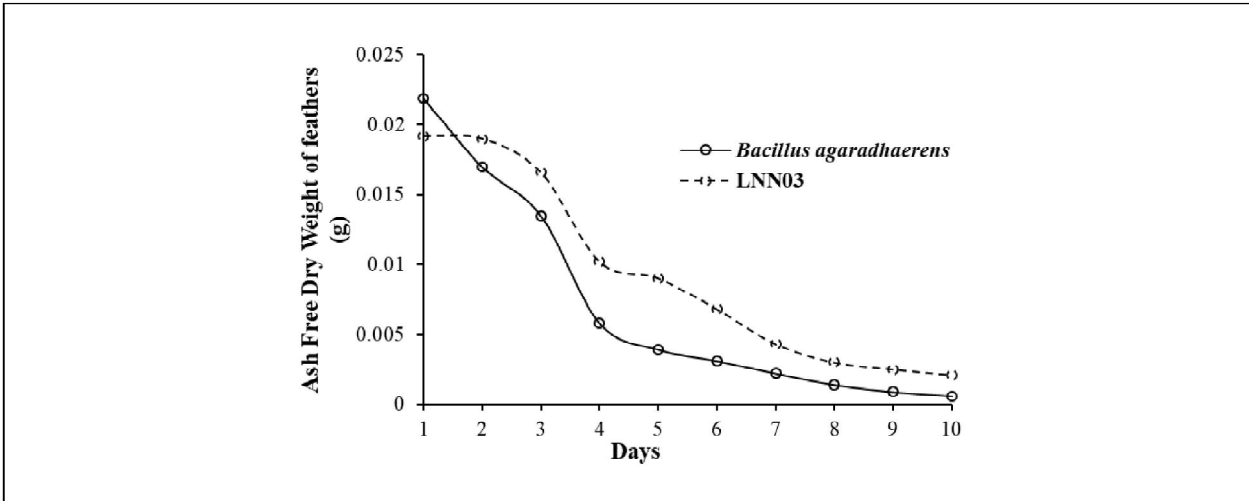


Figure 4: Profiles showing changes in ash free dry weight of chicken feathers during the assay inoculated with *Bacillus agaradhaerens* (LNC06) and LNN03 from Lake Nakuru for 10 days

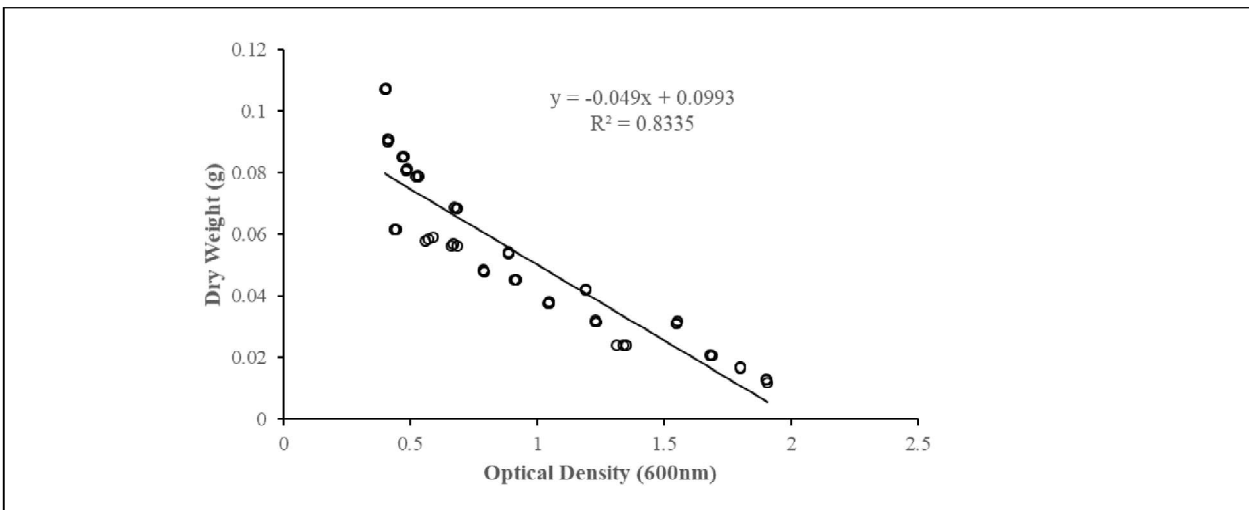


Figure 5: Relationship between optical density (600 nm) and dry weight of chicken feathers

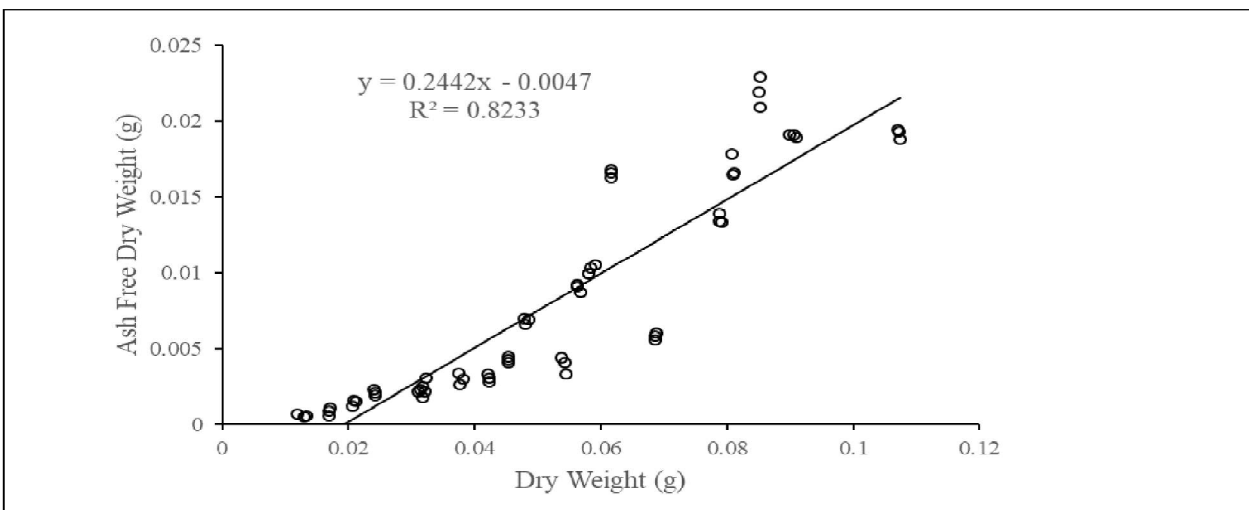


Figure 6: The relationship between dry weight and ash free dry weight of chicken feathers

4. Discussion

The northern and central part of Lake Nakuru had the highest number of flamingos during the time of sampling which coincidentally had isolates capable of degrading flamingo feathers. The presence of these isolates was due to the availability of substrate for bacterial metabolism resulting from flamingo waste and the entry of municipal sewage into the lake. *Bacillus agaradhaerens* which was faster in the degradation of feathers was isolated from degrading flamingo feathers from the shore. *Bacillus* species have been reported to produce keratinolytic enzymes responsible for the bioconversion of keratin in feathers (Zerdani et al., 2004). *Bacillus licheniformis* a soil inhabitant bacterium has been found to degrade feathers at an optimal temperature of 50 °C in 10 days (William et al., 1990). In contrast, the isolated Gram positive *Bacillus agaradhaerens* was found to grow in natural media without any special requirements except for alkaline pH of 10 and a temperature of 35 °C. It is able to breakdown the recalcitrant keratinous structure of feathers with less energy input to simple form that is rich in utilizable proteins that can be used by other animals as protein supplement to improve their nutritional needs (Bo Xu et al., 2009).

The resistance of keratin to degradation by protease enzymes in microorganisms has been attributed to their complex helical structure and disulphide bonds (Onuoha and Chukwura, 2011). Isolate LNN03 had positive effect in degradation of protein and keratin thus indicating its potential for production of protease enzyme. Despite it having keratinolytic properties, it was not a *Bacillus* species as indicated by its cocci structure and the absence of spores. Reports have identified *Bacillus megaterium* as an efficient keratin degrader and noted the ability of *Bacillus* sp to cause considerable degradation in the first 72 h of incubation (Sekar et al., 2015).

Chicken wastes particularly feathers are poorly disposed in landfills and combustion forms their major way of disposal causing an adverse effect on the environment (Joshi et al., 2007). Enzymatic hydrolysis of feather wastes or dead chicken could be a safer method of recycling these organic materials into a form that can be utilized by animals as protein feeds supplements. *Bacillus agaradhaerens* optimal growth at a temperature of 35 °C means that it requires less energy in a controlled process for efficient and faster degeneration of chicken feathers. This bacterium is economically advantageous in feather degradation than *Bacillus licheniformis* due to the low temperatures required for its optimal growth.

Previous studies on bacterial degradation of feathers have concentrated on isolation of these microbes from soil, feather waste digester and landfills (Williams et al., 1990; Zerdani et al., 2004 and Joshi et al., 2007) but none on flamingo feathers. Efficient feather degradation by *Bacillus agaradhaerens* in a dozen days confirms the potential use of this microbe for conversion of keratin to forms utilizable by animals in their feed supplements.

5. Conclusion

This study identified native microorganism from flamingo feathers in Lake Nakuru as being able to degrade proteins and chicken feathers. The isolate was identified as *Bacillus agaradhaerens* (LNC06). It grew well at a pH of 10 and temperature of 35 °C requiring minimal nutrients for effective degradation of feathers. Therefore, the newly isolated *Bacillus agaradhaerens* shows potential for use in biotechnological processes that involves feathers hydrolyses and can be used in the production of animal feeds because of the breakdown of keratin to amino acids. This application is also environmentally friendly.

6. Recommendation

This study isolated and identified *Bacillus agaradhaerens* from flamingo feathers with the ability to degrade chicken feathers. However, there is need to carry out further studies to establish suitability of amino acids from chicken degraded feathers as feed supplement. Further work should be done on *Bacillus agaradhaerens* in the evaluation of their keratinolytic enzymes as catalysts in biotechnology, bioremediation and feather hydrolytic reactions.

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