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Isolation and Identification of Feather Degrading Bacteria from Poultry Waste Dump site along Enugu - Port Harcourt Express way, Gariki, Awkunanaw, Enugu.

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ABSTRACT

The investigation was aimed at isolating and characterizing the culturable feather degrading bacteria from soils of the poultry waste dump site along Enugu - Port Harcourt express way by Gariki, Awkunanaw, Enugu. The bacteria that were isolated were tested for their capability to grow on feather meal agar (FMA). The proteolytic bacteria were tested for feather degradation and were further identified according to their morphological and biochemical characteristics. The isolate were gram positive, rod shaped and spore-former and were able to utilize glucose, sucrose and lactose. They were also catalase and oxidase positive. They showed typical characteristics of *Bacillus* sp., thus were from the *Bacillus* genus. This *Bacillus* strain is therefore a promising strain for the management of chicken feather waste through biotechnological processes.

1.0 Introduction

The day by day increase in consumption of meat received from chicken is causing harsh effect to environment, as the waste from the chicken birds; more particularly the feathers are not properly treated especially in third world countries. In nature, the deterioration of feather is slow because of fibrous protein called keratin, a cytoskeletal coiled-coil structural protein with a high degree of disulfide cross-link (Latkowski and Freedberg1999); it is the most abundant macromolecular component of feathers. Its thermal stability and the stringency of keratin confers on it the difficulty of degradation thereby causing environmental problem (Ezenwali, 2022).

Recycling of feathers can provide a cheap and alternative way of reducing the environmental challenge and at the same time offer a window for the production of feed ingredient like protein to aid feed millers and farmers involved in milling to produce feed for poultry and livestock. However, poor digestibility of keratin is a problem in recycling which recent researches have indicated positive result through biodegradation by microorganisms. These microorganisms are known as keratinases and have keratynolytic properties thus can effectively degrade keratinous waste (Sharma and Gupta 2010).

Many research works have implicated the following genera of bacteria as keratinolytic bacteria capable of degrading feathers: *Bacillus subtilis* AMR (Mazotto *et al.,* 2010; Korkmaz *et al.,* 2004; Ionata *et al.,* 2008; Rai *et al.,* 2009; Shrini-

vas and Naik 2011; Ramnani *et al.,* 2005), Fervidobacteriumislandicum (Nam *et al*., 2002), Elizabethkingiameningoseptica KB042 (Nagal and Jain 2010), Strain *Chryseobacterium*sp. kr6 shown to be a useful microbial agent in the hydrolysis of poultry feathers (Ezenwali, 2021). Pseudomonas aeruginosaKSl (Sharma and Gupta 2010) and Actinomycetes such as Streptomyces sp. (Mabrouk 2008; Syed *et al.,* 2009; Shilpa *et al.,* 2014) and fungi such as Chrysosporiumtropicum (Marathai *et al.,* 2011), Trichodermaatroviridae (Cao *et al.,* 2008), Doratomycesmicrosporus (Gradisar *et al.,* 2005); Paecilomycesmarquandii (Anbu *et al.,* 2007); Scopulariopsisbrevicaulis (Rodrigues *et al.,* 2008); Alternaria, Paecilomyces, Penicillium , Curvularia and several Aspergillus sp. (Thomatti and Peramachi 2012).

Keratin, apart from being one of the most widely generated soil/water wastes; it stays in the dump sites/waste streams for long occupying large volumes of space due to slow degradation. Most feather waste are disposed through land filling or burnt which involves huge expense and can cause contamination of air, soil and water. Utilizing poultry feathers as a fermentation substrate in conjunction with keratin degrading microorganisms and enzymatic degradation may be better alternative to improve nutritional value of poultry feathers and reduce environmental waste (Veslava Matikeviciene *et al.,* 2009).

In view of above, the present study is aimed to isolate, identify and characterize the kearatin degrading microorganisms from poultry farm soil in Agbani of Enugu State in order to study their feather degradation potential for biotechnological purposes.

2.0 Material and Method

The experiment was carried out at the laboratory of Applied Biochemistry/Microbiology in Enugu State University of Science and Technology (ESUT) and Brain-Phosphorylationship Scientific Solution Services, 5thFloor, Right Wing, No. 9 Ogui Road Enugu, Enugu State, Nigeria. ESUT is located in Nkanu West Local Government Area of Enugu State.

2.1 Collection of samples

Feather samples were collected from a poultry waste dump site along Enugu - Port Harcourt express way by Gariki, Awkunanaw, Enugu.

The samples were collected according standard microbiological procedures (Cappuccino, J. and Sherman. N. 1993; Steubing 1993) during the month of November 2022. In brief, about 4 gram of samples were collected in dark polythene bagsusing sterile spatula and immediately transported to the Applied Biochemistry Laboratory of ESUT and stored at 4°Cor further analysis.

2.2 Preparation of substrate

White chicken feathers were used in this study to prepare the pure feather meal powder. They were first washed extensively under tap water to remove blood and any dust particles. This was followed by washing with 0.1% Triton X-100 and then abundantly with distilled water. All the materials were later oven-dried at 75°C for 8 hours. The dried feather materials were chopped into pieces not exceeding 1.5cm in length, and then milled to 60-mesh particle size. The powders were kept at room temperature and used for further studies. For the primary screening, skimmed milk agar was used comprising of 5.0g/l peptone, 3.0g/L yeast extract, 100 ml/l UHT skimmed milk, and 12g/l agar.

2.3 Preparation of media and sterilization

The media was constituted using the following salt medium shown in Table 1:

Table 1: Composition of media mixture

Constituent	Concentration (g)
K2HPO4	1.8g
NH4CL	-4.0g
MgSO4.7H20	0.2g
Nacl	0.1g
FeSo4.7H20	0.01g

It was sterilized at 121^{0}C for 15minutes using an autoclave

2.4 Isolation of microorganisms

Soil sample of 1g was cultured on mineral salt medium along with defatted feathers as the sole carbon source and incubated for 3 days in a rotary shaker. The medium was tested for degradation of keratin in the feather using ninhydrin solution. A volume of 0.5ml of the medium was inoculated into agar plates to observe the growth of possible keratinolytic microbes.

The plates were incubated at 37°C for up to three days. Distinct colonies, observed using morphological features, were selected, isolated, and purified on keratin agar to obtain pure cultures. The pure cultures were identified by means of taxonomic schemes and descriptions (Buchanan and Gibbons, 1974).

2.5 Inoculation and Incubation of the Media

The media after sterilization and cooling, isolate suspected to degrade keratin was inoculated into the media and was incubated using rotary shaker incubator.

2.6 Identification of Bacteria species

The inoculated plates were examined and different colonies observed using physical and morphological differences were sub-cultured to obtain pure cultures. The pure cultures gotten were sub-cultured into an agar slant in bijour bottle and thereafter kept in refrigerator at 4^{0}C to serve as stock culture. The isolates were identified and characterized using different biochemical test such as; Gram stain, catalase, coagulase, oxidase, Indole, sugar fermentation test and molecular analysis.

2.6.1 Biochemical Characterization

2.6.1.1 Gram Stain:

One loopful of each of the organisms that were isolated from the sample were placed and smeared on microscopic slides with a sterile wire loop. The organisms were allowed to air dry and afterwards were heat fixed. Then 5 drops of crystal violet were added on the organisms and were allowed to stand for 1 minute and were washed off with water afterwards. Then 5 drops of iodine solution was added and allowed to stand for 30sec and washed briefly with water. The slides were then tilted and alcohol was used to run on the organisms to decolorize the purple colour. They were immediately washed with water for 5 seconds. 5 drops of safranine were added and were left to stand for 1 minute and were briefly washed off with water. The organisms on the various slides were then examined under the microscope using X100 objective lens (oil immersion). On examination result showed that the organisms retained the purple color of crystal violet proving that the organisms are gram positive.

2..6.1.2 Catalase test

One loop full of the organisms was transferred to a clean glass slides using sterile wire loop. Two drops of 3% hydro-

gen peroxide (H_2O_2) was put on the slide and were mixed. Positive result was taken note of by the production of bubbles.

2.6.1.3 Coagulase test

A loopfull of each of the isolates was smeared on a clean glass slide. Blood plasma was transferred on the smear using a sterile wire loop and and a clumping structures were observed within 10 seconds, this proves the isolate was coagulase positive.

2.6.1.4 Citrate Test

A small amount of the culture growth was picked from the nutrient agar plates, using glass rod and lightly inoculated on Simon citrate agar slants and incubated at 37^0c for 24 hours. A positive test was indicated by the development of a blue colour in the medium. While in a negative reaction the medium retained its original pale green color after incubation (Cappuccino and Sherman, 2005).

2.6.1.5 Indole test

Total of 5 drops of Kovac's reagent were added directly into the tubes. Tryptophanase breaks down tryptophan to release indole, which on reacting with cinnamaldehyde produced a blue-green compound; note that the absence of enzyme results in no color production, which is indole negative.

2.6.1.6 Oxidase test

Filter papers were soaked with tetramethyl-p-phenylenediamine-dihydrochloride substrate. The filter papers were moistened with sterile distilled water. The isolates were collected with a wooden stick and smeared on the filter paper (Shields and Cathcart, 2010).

2.6.1.7 Motility test

With a sterile straight needle, the colony of a young (18 to 24hour) culture growing on agar medium wassingle stabbed down the center of a tube to about half of the medium. Then it was incubated at 35-37^{o}C and examined daily for up to 7 days. Motile organisms spread out into the medium from the site of inoculation. Non motile organisms remain at the site of inoculation.

2.6.1.8 Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment five sugars with production of acid and gas. Peptone powder (15g) was dissolved in 100ml of distilled water with 0.5g of bromothylmol blue. Durham tubes were inserted inversely into all the test tubes. Then 9ml of the mixed peptone medium was added to each of the test tubes and sterilized in an autoclave at 121oC/15psi for 15 mins. The sugars were prepared 54 by adding 0.5g of the sugars to 5ml of sterile distilled water. The sugar was purified using membrane filter to filter the dissolved sugar solutions. Thereafter, 1ml of each sterile sugar solution was added into the 9ml of sterile peptone water to form sugar fermentation medium. Subsequently, a loopful of each isolate was inoculated into the fermentation medium and incubated at 37oC for 24 h. Presence of acid (yellow coloration) and gas (bubbles in Durham tubes) was observed and recorded.

3.0 Results and Discussion

3.1 Isolation and Separation of Bacteria Isolates

Bacillus sp. was found and was able to grow on medium containing feather meal as sole carbon and nitrogen source. The strain produced clearing zones when tested for proteolytic activity on milk agar (Figure 1 below).

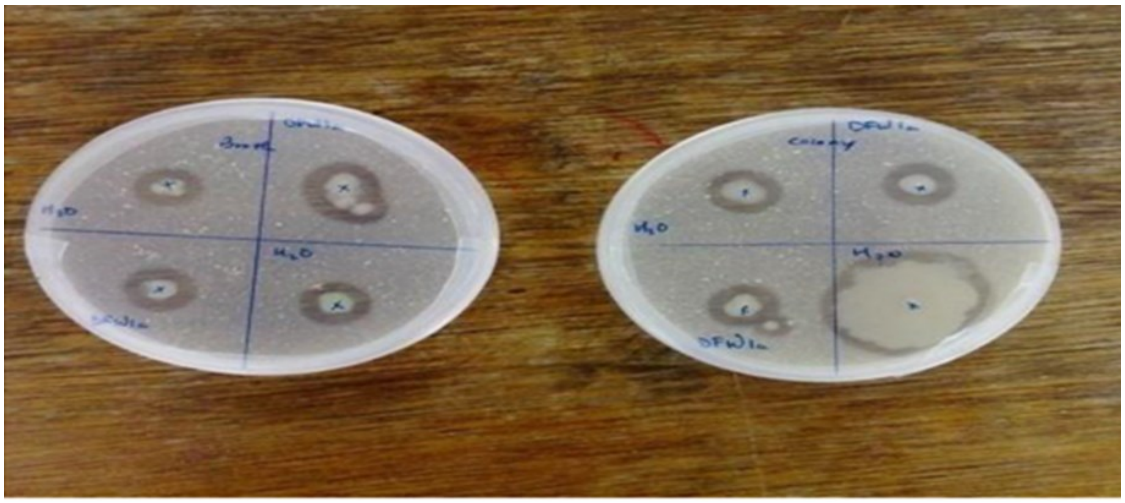

Figure 1: Distinct clearing zones in milk agar plates shown by the keratinolytic bacteria

Soil sample collected from feather dumping site was cultured on mineral salt medium along with defatted feathers and incubated for 3 days in a rotary shaker. The medium was tested for degradation of keratin in the feather using ninhydrin. It was observed that on addition of ninhydrin to a known volume of the medium containing the sample (culture) with addition of heat, the medium turned purple showing the presence of amino acid which is a by-product of keratinase degradation of keratin in the feather. The culture was sub-cultured in a solid medium (nutrient agar) this is to observe the growth of microorganisms responsible for the degradation of the feather. Pure isolate of the microorganism isolated was then used to set up a fresh mineral salt medium to confirm keratinoltic ability of the microorganism. Identification of the microorganism was done using biochemical methods such as gram staining, catalase test, indole test etc. After the identification process bacillus sp was obtained.

3.2 Identification of Keratinolytic Bacteria

The identification of the keratinolytic bacteria was based on morphological, cultural and biochemical tests comparing the data with standard species (Hoqn *et al.,* 2005). Morphological and physiological characteristics of the bacteria were compared with the Bergey's Manual of Systemic Bacteriology.

The results are shown in Table 1. All isolates were Gram positive, rod shaped and spore-former, and were able to utilize glucose, sucrose and lactose. They were also catalase and oxidase positive. The organisms were motile, unable to utilize citrate and all were able to reduce nitrate to nitrite. All isolates showed typical characteristics of *Bacillus* sp.

Table 2. Morphological, physiological, cultural and biochemical characteristics of isolate

Experimental details	Observations
Shape	Rod and Spore former
Endospore formation	+
Motility	+
Gram stain	Positive
Anaerobic growth	-
Biochemical Characteristics	
Oxidase	+
Catalase	+
Citrate hydrolysis	-
Urea hydrolysis	+
Fermentative test	Heterofermentative
Lactose	+
Saccharose	+
Glucose	+
Fructose	+
Galactose	+
Colony characteristics	
Growth	Rapid
Shape	Irregular
Surface	Smooth
Margin	Entire
Colour	Cream/white
Elevation	Flat/convex
Consistency	Buttery/viscous
Opacity	Opaque

Key: +, Positive; -, negative

Collectively these characteristics indicated that the isolate was of genus *Bacillus*. Keratinolytic bacteria, particularly from the genus *Bacillus* have been isolated from the plumage and bird feathers (Burtt and Ichida, 1999; Ichida *et al.,* 2001), feather waste processed by fermentation (Williams and Shih, 1989; Williams *et al.,* 1990) or composting (Kim *et al.,* 2001). In bacteria, feather keratin-degrading abilities have been observed mostly in strains of *Bacillus licheniformis* (Burtt and Ichida, 1999; Lin *et al.,* 1999), less frequently in populations of *Bacillus pumilis, Bacillus cereus and Bacillus subtitis* (Kim *et al.,* 2001).

The production and processing of poultry involve killing, defeathering, eviscerating and cleaning that lead to the generation of wastes. Coker *et al.,* (2001) showed that these wastes can affect water, land and air qualities if proper practices of management are not followed. The findings of the present study support the study hypothesis that the deposited poultry wastes serve as a reservoir for the growth and multiplication of bacteria.

The colonization of pathogenic bacteria in these dumping sites is highly hazardous to the environment. If these infectious poultry wastes are discharged into lakes, ponds and/or other drinking water resources it can harm aquatic life and jeopardize the quality of our drinking water. In addition, the nuisance odour arises from these materials has also an impact on greenhouse gas emissions associated with global climate change. These waste materials need prompt disposal system or should be utilized as an alternative source. Several ways of disposing methods are followed worldwide such as burial, rendering, incineration, composting, feed for livestock, fertilizer or source of energy as recommended by Hazard Analysis Critical Control Point (HACCP) guidelines (Pope and Cherry 2000) and Environmental, Health, and Safety Guidelines for Poultry Production Guidelines (WBG 2007).

4.0 Conclusion and Recommendation

Bacillus sp, a bacteria presenting high keratinolytic activity was isolated. Considering that feather protein has been showed to be an excellent source of metabolizable protein (Klemersrud*et al.,* 1998), and that microbial keratinases enhance the digestibility of feather keratin (Lee *et al.,* 1991; Odetellah*et al.,* 2003), thiskeratinolytic strain could be used to produce animal feed protein in addition to the biodegradation of poultry wastes, including composting (Korniłłowicz-Kowalska and Bohacz, 2011). It will also prevent ecological hazards resulting from keeping those wastes on waste

dumps.

Present study has some limitations. There is limited availability of resources and laboratory facilities. Investigations with advanced techniques like PCR, molecular sequencing and flow cytometry would be carried out later to reveal more scientific information.

It is recommended that using the isolate, *Bacillus sp.* presents potential biotechnological use in processes involving keratin hydrolysis.

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