Isolation and Identification of Chicken Feather Degrading Organisms from Soil Sample

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Abstract: - The daily consumption of chicken increases annually, as they are one of the cheapest and healthiest sources of protein. About 3 billion pounds of chicken feathers are generated every year in the world with a great percentage disposed as waste. Feathers can be disposed by incineration, controlled land filling, etc but they can conceive infectious agents and need special requirements from reaching the ground water. 90% of feather biomass constitutes primarily β-keratin, insoluble protein extensively cross-linked by disulfide bonds. Keratin is highly resistant to microbial degradation due to the tight packing of the protein chain either in alpha helix or beta sheet structures and their linkage by cystine bridges that have a high degree of cross linkages by disulfide bonds, hydrogen bonding and hydrophobic interactions. The efficient degradation takes up to many years. Thus, an appropriate technique is needed to overcome this problem. . Biodegradation is an enzymatic degradation involving some microorganisms including bacteria, fungus. The enzyme involved in the degradation of keratin is called as keratinases. The work presented here is to isolate and identify chicken feather degrading organisms from soil sample. Seven soil samples were collected from different sites in and around Coimbatore and Tirupur district. Soil samples were serially diluted and different types of bacteria were isolated on nutrient agar medium and different fungal species were isolated on potato dextrose agar medium. The isolated bacterial species were then screened on skim milk agar and keratin agar medium. After identification of the bacterial and fungal species, they were screened on raw feather broth medium incorporated with chicken feathers. Finally, the organisms showing efficient degradation were chosen and used in the feather compost. The final component of the feather compost can be used as value added products like bio-fertilizers and animal feeds. This technique can be used to prevent feather accumulation in dumping sites.

Key Words: — ***Feather waste, keratin, keratinase, feather compost, value added products.***

I. INTRODUCTION

The daily consumption of chicken increases annually as they are one of the cheapest and healthiest sources of protein. About 3 billion pounds of chicken feathers are generated every year in the world with a great percentage disposed as waste. Feathers account for approximately 5-7% of the total weight of a mature chicken and feather waste represents a huge resource of protein. 90% of feather biomass constitutes primarily β-keratin, azelon, and insoluble protein extensively cross-linked by disulfide bonds, 70% of amino acids, high-value elements, vitamins, and growth factors (Sayali kodak *et al*., 2019).

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The enzyme involved in the degradation of keratin is known as keratinases. A number of feather degrading species of bacteria, actinomycetes and fungi have been used for the production of keratinase enzyme. Keratin can be degraded by keratinase produced by some species of bacteria such as *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Arthrobacter* sp, *Kocuria rosea* and fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Rhizomucor* sp, *Alternaria radicina*, *Absidia* sp, *Onygena* sp, and *Penicillium* sp.

The increased production of poultry industry results in a huge amount of waste that needs to be managed. Although a controlled utilization of such waste as soil fertilizer is feasible, some concerns are due to the high levels of oxygen demand, physical clogging of soil by fat accumulation, and presence of pathogens in dead, hatchery and litter compost. The major waste materials generated in poultry processing plants are feathers, soft meat, blood, deboning residue, and also dead on arrival. These materials are currently converted into meat and

bone meal, feather meal, blood meal and fats/oils by rendering process. Although these meals are a good source of protein, their utilization can be limited due to nutritional restrictions associated with losses of essential amino acids, and with high calcium, phosphorous and lysine content of the meat and bone meal.

Feathers can be disposed by incineration, which is one effective method to destroy conceivable infection agents. Feathers can also be disposed through burial and controlled land filling while special management is required to keep them from ground water.

The traditional feather processing methods such as chemical treatment and stem pressure cooking method could convert feathers into animal feeds, but the processes need a large amount of energy and some amino acids are destroyed during treatment.

Although feathers can be utilized as materials in different fields, a large amount of feathers are still released into the environment without proper treatment. Feathers have become one source of pollutant because of their recalcitrant nature.

Untreated feather waste can sustain many pathogenic microorganisms and emit various pollutants such as nitrous oxide, ammonia, and hydrogen sulfide, which are a threat to the environment and people's health. Therefore, converting feathers into value-added products using economic methods is of great interest to many researchers.

II. MATERIALS AND METHODS

Collection of Soil Samples:

Seven different types of soil samples were collected from in and around Coimbatore and Tirupur district. The seven soil sample (A, B, C, D, E, F, G) were indigenous to locations:(A) Soil from Poultry Farm – 1,(B) Soil from Poultry Farm – 2,(C) Soil from Chicken Cage,(D) Fertile soil,(E) Chicken faeces, (F) Soil from Dump site (Waste), (G)Soil from Agricultural Land.
The soil samples were collected at a depth of 3-5cm, in a sterile container. The samples were sealed properly, labeled and transported to the laboratory. All the samples were processed within 24 hours of collection.

Collection of Chicken Feathers:

Chicken feathers were collected from a local slaughter-house and local poultry processing waste site.

Surface Sterilization of Feathers:

Fig.1. Surface sterilization of feathers

The raw feather was taken and cut with small size. The pieces were cleaned with tap water to (remove dust particles). Then, surface sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again washed with distilled water and dried at 45°C for 24 hours in circulating hot air over. The weighed about 1g each and used for both test and control experiment. (Tamil kani et al., 2012).

Isolation of Fungi:

The samples were subjected to serial dilution to get diluted and inoculated on to the sterile petri dishes which contain Potato Dextrose Agar. The serial dilution spread plate method was followed to isolate fungus from soil samples. 1g of the effluent soil samples was added to 100 ml of distilled water and was serially diluted up to 10-7. The fungi were isolated by spread plating 0.1 ml of each of the dilution on potato dextrose agar plates.

The plates were kept for incubation at room temperature for 4 days to obtain colonies. The individual colonies were picked upon the basis of their characters such as shape, surface, appearance and colour. They were subcultured again to potato Dextrose Agar and Rose Bengal Agar.

Fungus was identified on the basis of colony characteristic (macroscopic), microscopic view Lacto-phenol cotton blue was used to stain the fungi for microscopic view and for the spore's arrangement (Kartikey kumar gupta et al., 2017).

keratin Degradation By Fungi:

The isolated fungi from the poultry soil have the ability to degrade keratin and producing a keratinase enzyme. Potato dextrose broth was prepared based on the composition and sterilized at 121°C for 15 minutes under 15 lbs pressure. After sterilization, add 1gm of pre-weighed surface sterilized feathers

aseptically. Inoculate the isolated fungal colonies in the broth and incubate the plates at room temperature for up to 1 month.

Isolation of Bacteria:

1gm of poultry waste was serially diluted in order to reduce the initial number of microorganisms. This dilution was then inoculated into basal feather broth. Feathers were washed, dried and hammer milled prior to being added to the medium.

The medium was sterilized by autoclaving. All incubations were done at 37°C with shaking at 120 rpm in a controlled environment shaker. (Jai Godheja et al., 2013).

Screening of Protein Degrading Organisms:

Skim milk agar (Himedia) was prepared and the above dilutions were streaked on milk agar plates for testing the caseinolytic activity of the organism. Bacteria were inoculated onto plates and incubated at 37°C for 24 h. Strains that produced clearing zones in this medium were selected. (Jai Godheja et al., 2013).

Preparation of Keratin Substrate:

Raw feather medium was used for the identification of feather degrading microorganisms contained the following constituents.

Sodium chloride (Nacl) - 0.5, Ammonium Chloride (NH4Cl) - 5.5, Di-potassium hydrogen orthophosphate (K2HPo4) - 0.3, Potassium dihydrogen phosphate (KH2Po4) - 0.4, Magnesium chloride Mgcl2 - 0.24, Yeast extract - 0.1, Agar – 15 gm, Distilled water - 1000 ml, pH - 7 .5

1000 ml of raw feather medium was prepared and autoclaved at 121°C for 15minutes. The sterile pre weighed small feather pieces were aseptically transferred into respective medium.

Screening of Keratin Degrading Organisms:

The colonies obtained from skim milk agar plates were transferred to keratin agar plates.

Characterization and Identification of Isolated Keratin Degrading Bacteria:

The bacterial isolates were gram stained and observed under a high power objective in light microscope. Motility test was performed to observe the morphology and motility of the cells.

The bacterial isolates were characterized biochemically by indole test, methyl red test, voges proskaeur test, simmons citrate test, catalase test, oxidase test, urease test, starch hydrolysis test, H2S production and carbohydrate fermentation test (glucose, sucrose, lactose and mannitol).

Sub culturing:

The organism screened with Keratin agar plates was subcultured by continuously growing the bacterium in basal broth medium (4 days at 37°C, 120rpm) and subsequently streaking on basal agar medium (2% agar, 2 days 37°C). (Jai godheja et al., 2013).

Analysis of Keratin Degradation:

Raw feather broth was used for fermentation of the feather degrading microorganisms contained the following constituents.

Sodium chloride (Nacl) - 0.5, Ammonium Chloride (NH4Cl) - 5.5, Di-potassium hydrogen orthophosphate (K2HPo4) - 0.3, Potassium dihydrogen phosphate (KH2Po4) - 0.4, Magnesium chloride Mgcl2 - 0.24, Yeast extract - 0.1, Distilled water - 1000 ml, pH - 7 .5

1000 ml of raw feather broth were prepared and autoclaved at 121°C for 15minutes. The sterile pre weighed feather pieces were aseptically transferred into respective broth. A loopful of bacterial culture isolated from the soil samples were inoculated into respective medium.

One 250ml of flask containing only the feather was maintained as control. These flasks were incubated at 37°C for 30 days and observed after the following days for degradation of chicken feathers. (Tamil kani et al., 2012).

Preparation of Feather Compost:

The bacterial and fungal species that have the ability to degrade chicken feathers efficiently were identified. The soil sample for the compost was collected from our college campus and it was sterilized at 121oc for 15 minutes under 15 lbs pressure. 500 gram of soil sample was taken in a sterilized container and 5 grams of chicken feathers were added.

They were mixed well with a sterile spatula. Then 100ml of Bacillus sp, Bacillus licheniformis and Aspergillus niger broth cultures were added into the mixture of soil and chicken feathers. The feathers were kept for degradation for 30 days, respectively. The sterile water was sprinkled and mixed periodically.

III. Results And Discussion

A. Soil Sample Collection

Totally seven soil samples were collected from different locations in and around Coimbatore and Tirupur districts was mentioned in Figure 2.

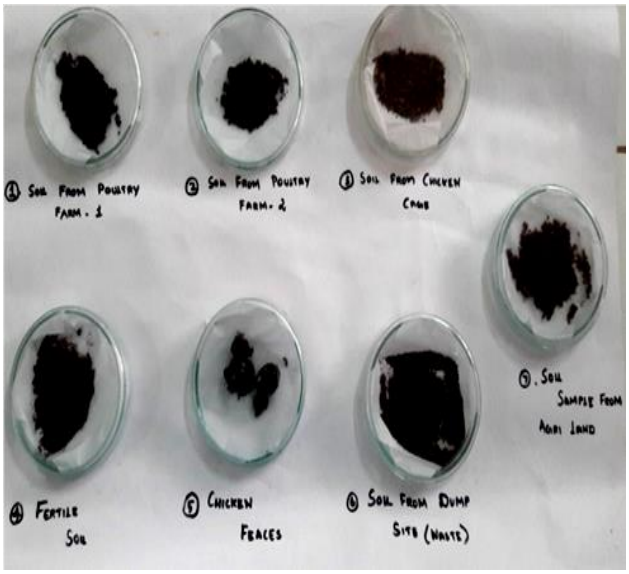

Fig.2. Soil sample collected from various regions

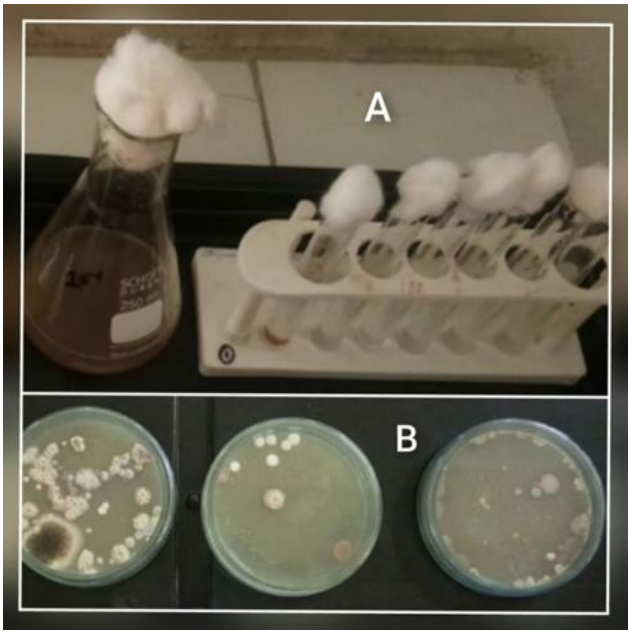

Fig.3. A) Serial dilution, B) Plating on SDA

Different types of fungi were isolated from the serially diluted plates (Figure 3). They were subcultured and grown on rose bengal medium. After 48 hours of incubation at room temperature, the fungi were identified by lacto phenol cotton blue staining. The isolated fungal species were identified as

- *Aspergillus niger* (figure 4)

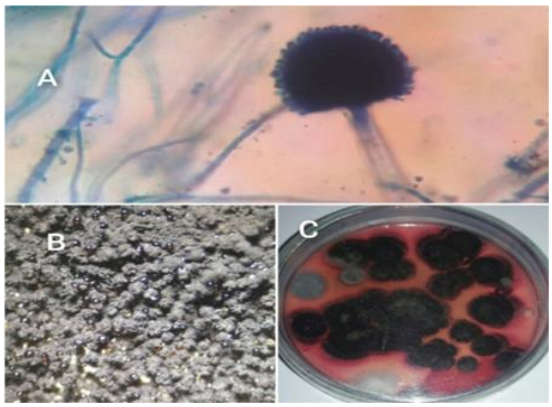

Fig.4. A) LPCB staining, B) Stereomicroscopic image, C) *Aspergillus niger* in rose bengal agar plate

- *Aspergillus flavus* (figure 5)

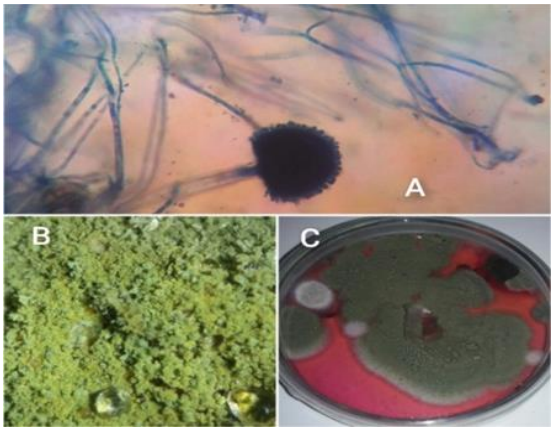

Fig.5. A) LPCB staining, B) Stereomicroscopic image, C) *Aspergillus flavus* in rose bengal agar plate

- *Penicillium* sp (figure 6)

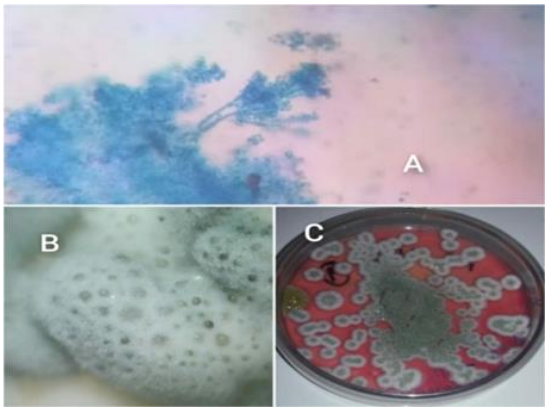

Fig.6. A) LPCB staining, B) Stereomicroscopic image, C) *Penicillium* sp in rose bengal agar plate

- *Fusarium* sp (figure 7)

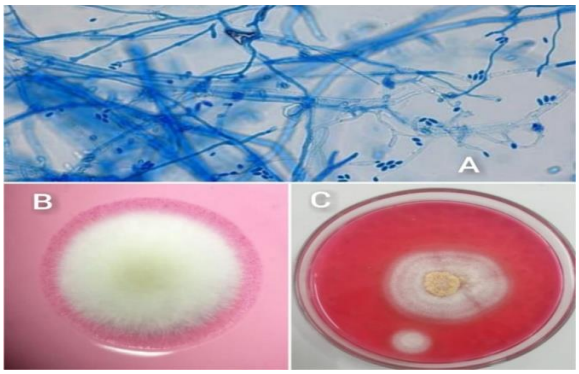

Fig.7.A) LPCB staining, B) Stereomicroscopic image, C) *Fusarium* sp in rose bengal agar plate

B. Feather Degradation by Fungi

The conical flasks inoculated with fungi were observed the following weeks for degradation process (Figure 8). The medium inoculated with Aspergillus niger showed effective degradation of chicken feathers after 30 days when compared to other fungal species (Figure 9).

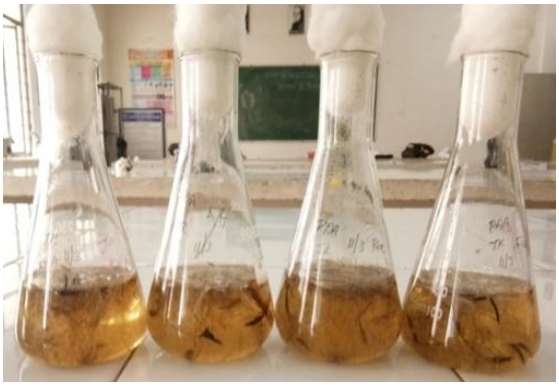

Fig.8. Potato dextrose broth before incubation

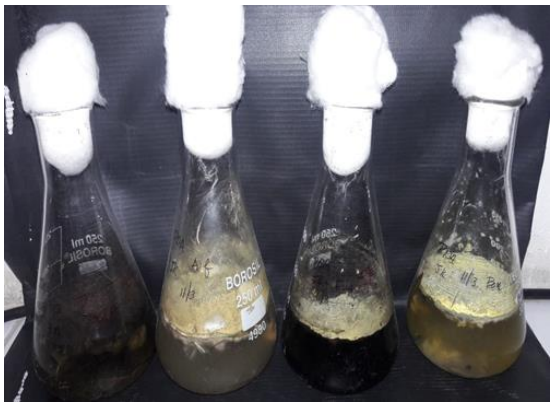

Fig.9. Potato dextrose broth after incubation

C. Isolation of Bacteria

After serial dilution, colonies from the dilution 10-5, 10-6 and 10-7 were selected for further evaluation (Figure 10).

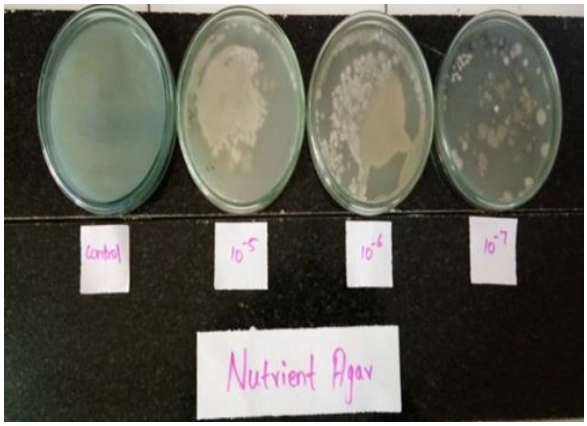

Fig.10. Isolation of bacteria on nutrient agar by serial dilution method

D. Screening of Protein Degrading Organisms

The colonies isolated from the serially diluted plates were streaked onto skim milk agar plates and incubated at 37°c for 24 hours. The following day, skim milk agar plates were observed for zone formation. Zone forming colonies from the skim milk agar plates were selected and processed further (Figure 11).

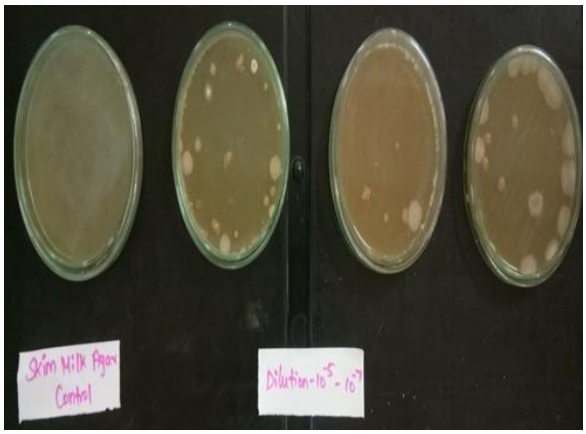

Fig.11. Screening of protein degrading organisms

E. Screening on Keratin Substrate

Zone forming colonies from the skim milk agar plates were further streaked onto keratin agar plates. The plates were incubated at 37°c for 24 hours While observing on the next day, bacterial growth was seen (Figure 12).

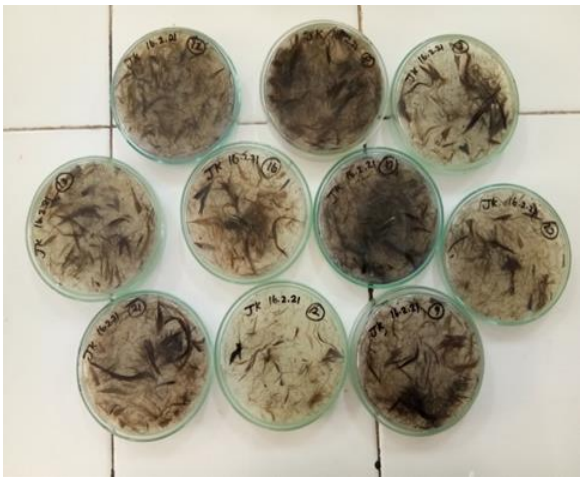

Fig.12. Screening on keratin agar plates

F. Characterization and Identification of Isolated Keratin Degrading Bacteria

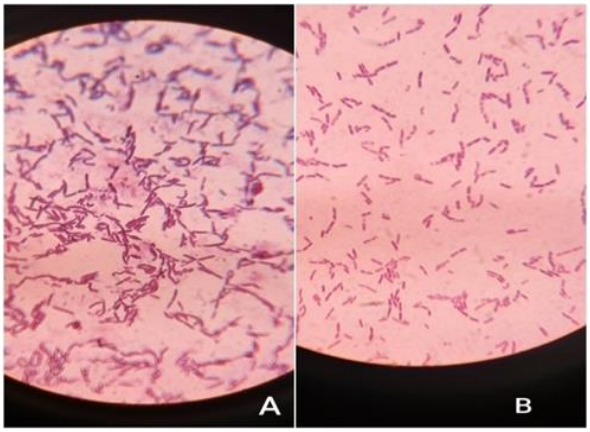

Fig.13. Gram staining A) *Bacillus* sp, B) *Pseudomonas* sp

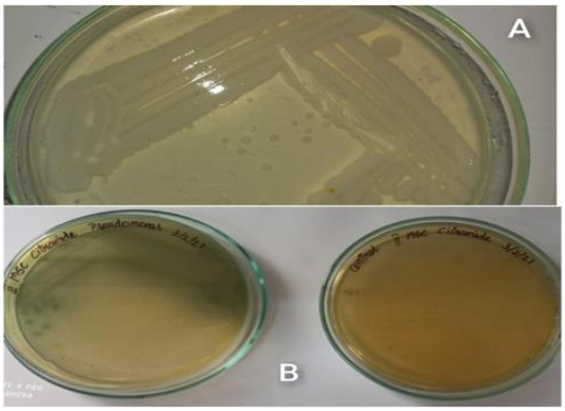

Fig.14. Cultural characteristics A) *Bacillus* sp in nutrient agar plate, B) *Pseudomonas* sp in cetrimide agar plate

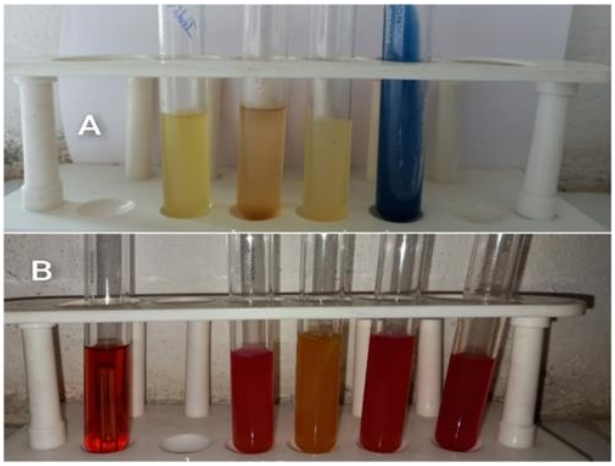

Fig.15. Biochemical tests A) *Pseudomonas* sp IMVIC test, B) *Pseudomonas* sp Carbohydrate fermentation test

Gram staining of the isolated bacteria showed **violet colored rod shaped and pink colored rod shaped** organisms (Figure 13). Based on the colony morphology on the selective medium (Figure 14) and biochemical tests (Figure 15), the organisms were identified as

- ***Bacillus* sp**
- ***Pseudomonas* sp**
- ***E. coli***

G. Preparation of Raw Feather Broth

The isolated bacterial colonies were inoculated onto the raw feather broth and observed the following day. Among the other bacterial species, ***Bacillus* sp** showed degradation of feathers after 30 days (Figure 16).

Fig.16. Raw feather broth

H. Feather Compost

The feather compost was observed the following weeks after inoculation of cultures (Figure 17). Moisture content was maintained by sprinkling water periodically. The compost was turned in a regular basis. The degradation process of chicken feather has been going on for about 22 days. The compost is kept for further degradation and evaluation (Figure 18).

Fig.17. Preparation of feather compost

Fig.18. Compost after 20 days

IV. DISCUSSION

Various types of microbes were isolated from soil sample collected from local poultry processing site. Bacterial species such as Bacillus sp, Pseudomonas sp and fungal species such as Aspergillus niger, Aspergillus flavus, Penicillium sp and Fusarium sp were isolated. Among the bacteria, Bacillus sp showed degradation of chicken feather waste whereas in fungi, Aspergillus niger showed efficient degradation of the chicken feathers. Further, the organisms were used in the preparation of feather compost and observed for degradation. After further degradation, the chicken feathers were analyzed further.

V. SUMMARY

Soil sample was collected from various sites and serially diluted to minimize the microbial load. Different types of microorganisms such as bacteria and fungi were isolated. The isolated fungal species were identified using lacto phenol cotton blue staining. The isolated bacteria were then streaked on skim milk agar to identify protein degrading organisms. The organisms that produced clear zone on skim milk agar were then inoculated on keratin medium. The bacterial species were identified based on gram staining, cultural characteristics on selective media, colony morphology and biochemical tests.

Then the fungi were inoculated in potato dextrose broth along with chicken feathers for degradation process. Similarly, bacterial species were also inoculated in raw feather broth for degradation process.

The isolated fungal species Aspergillus niger sp inoculated on potato dextrose broth with feathers showed efficient degradation in the period of one month. Bacteria such as Bacillus sp and Pseudomonas sp started showing progress in degradation of chicken feathers within the period of 32 days.

VI. CONCLUSION

Chicken feathers are considered as pollutant because of their slow degrading ability in the soil. Thus, different types of microorganisms such as bacteria and fungi can be used to degrade chicken feathers quickly and efficiently. In my research, the fungus Aspergillus niger showed rapid degradation of chicken feathers when compared to bacterial species. These fungi can be used in composting chicken feathers and degrading them in an efficient way.

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