

Original Research Article

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Solid-state fermentation of chicken feathers by *Bacillus cereus* and study its potential as biofertilizer

Monisha Sankar, Yuvaraj M, Adaikkammai V, Nagalakshmi Kamaraj*

Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam, Maduranthagam-603308, Tamil Nadu, India

*Corresponding author; e-mail: knagalakshmiphd@gmail.com

Article Info

Abstract

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Poultry feather waste is the rich source of keratin protein which is highly insoluble and resistant to degradation by common proteolytic enzymes. Keratins are readily degraded by specific proteolytic enzyme keratinase. The proteolytic activity of *Bacillus cereus* was observed through clear zone formation in skimmed milk agar plate. The enzyme keratinase can be produced by keratinolytic microorganisms such as *Bacillus cereus* through solid-state fermentation using chicken feathers as the sole carbon and nitrogen source. Agricultural waste such as rice bran and trace salts are added along with chicken feather to increase the keratinase production. Feather hydrolysate was obtained after 72 hrs of incubation at 37°C which is a mixture of soluble proteins, peptides and free amino acids through degradation of 93% of feathers. Results revealed that the obtained feather hydrolysate contains indole acetic acid and act as potential biofertilizer through enhancing the germination rate, shoot and root development of *Vigna radiata*, *Abelmoschus esculentus* and *Oryza sativa*. This shows that chicken feathers can be recycled into valuable products such as biofertilizer, biofuel, dehairing agent and as detergent additive through sustainable method.

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Introduction

Poultry sector is growing tremendously in recent years due to increase in the demand of eggs and meat. It generates feather, blood, offal, hatchery waste and poultry litter as a by-product. Improper disposal of these waste products can cause environmental pollution (Muduli et al., 2019). Poultry feather wastes are abundant in keratin which on degradation generates soluble proteins (Dina. H. El Salamony et al., 2024), peptides and free amino acids. Amino acids have a

major role in growth of plants, uptake of nutrients and photosynthetic assimilation (Shakeelur Rahman et al., 2024). Degraded feather wastes are employed in bioplastic (Tesfaye et al., 2017), laundry detergent, biofertilizer, cosmetics and biofiber.

Chicken feather and Keratin

Chicken feathers contains nutrients such as 91% keratin, 8% moisture, 2% lipids (Muduli et al., 2019), phosphorus, potassium, calcium, magnesium, iron,

manganese, zinc, and copper (Gurav and Jadhav, 2013). Chicken feather contributes 5-7% of total body weight of an adult chicken. It is rich in hydrophobic amino acids like threonine, cysteine and arginine. Keratin contributes to the non-degradable nature of feather. They are not readily soluble in water, weak acids, strong alkalis, weak bases and strong acids excluding highly concentrated H_2SO_4 (Nurkhasanah et al., 2020).

Keratin is widely available natural polymer followed by chitin and cellulose. It is a protein present in feathers, nails, hair, beaks, wool, scales, hooves and horns (Bodde et al., 2011). Keratin is a semi-crystalline fibrous, low molecular weight insoluble protein. It contains about 18-20 types of amino acids. The primary structure of keratin is formed by binding of amino acids through peptide bonds. Hydroxyl group, carboxylic groups, amine group, thiol group and aromatic group of amino acids act as reactive site in keratin. It majorly consists of β -sheet arrangement. Hydrogen bond, disulfide bonds and hydrophobic interactions of polypeptides leads to supercoiling of macro-filament and micro-filament of keratin (Costa et al., 2012).

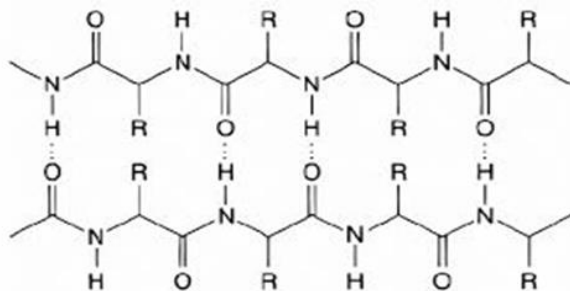


Fig. 1: Structure of β -keratin.

They are low degradable in nature. Hence, it is difficult to overcome impact of keratin material wastes in the environment. They can be degraded by methods involving high temperature and pressure which in turn produces elevated amount of sulphur and ammonia gases. These waste gases are dangerous to environment (Li et al., 2020). Continuous increase in the trend of dumping rate of keratin waste above certain threshold limit results in phosphorus runoff in nearby waterbodies and leaching of nitrate into underground water. The hydrolysis of keratins generates peptides, proteins and free amino acids by keratinolytic microorganisms due to the production of keratinase (Tamreihao et al., 2019). Physical and chemical treatment can degrade keratins accompanied by denaturation of heat sensitive amino acids due to prolonged exposure of high temperature and pressure.

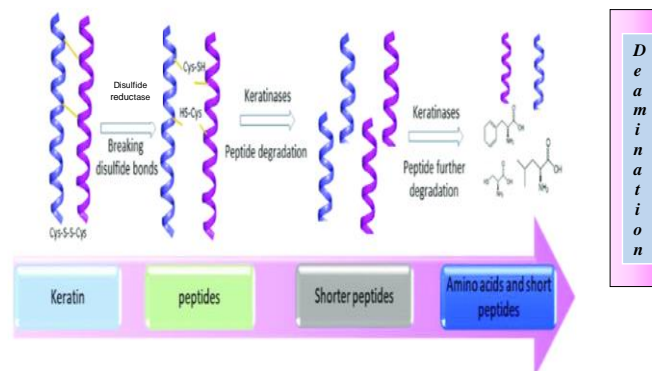


Fig. 2: Hydrolysis of keratin submerged fermentation.

Growing of microorganisms in a liquid medium containing nutrients is known as submerged fermentation. Powdered chicken feathers act as a substrate for keratinolytic bacteria. Microorganisms rapidly utilise nutrients from the free-flowing medium. The purification of product is easier. Sucrose, maltose, lactose acts as a carbon source while casein, yeast extract acts as nitrogen source. The nutrients are supplied either initially or at regular intervals. It is an expensive process. It provides highly purified product.

Solid-state fermentation

The solid substrates used in solid-state fermentation are wheat bran (Dina. H. El Salamony et al., 2024), rice bran, cow dung (Vijayaraghavan et al., 2014), agricultural wastes and so on. Solid substrates are the sole carbon and nitrogen source for bacteria, fungi, yeasts and other organisms. This fermentation is not an expensive process. Amylase is produced from cow dung substrate by *Bacillus cereus* (Vijayaraghavan et al., 2014). SSF is easily manageable with low energy requirement in pre- and post-processing. The products can be recovered easily accompanied by low effluent generation (Oliveira et al., 2006).

Bacillus cereus

Bacillus cereus is a rod-shaped gram-positive bacterium. It is a motile, spore forming facultative anaerobe or aerobic bacteria. The growth is observed in wide range of temperature ranges from 8-55°C. The spores are highly resistant to unfavourable conditions and rapid growth is observed on the availability of decomposable matter in the soil (Callegan et al., 1999). It is reported that thermostable keratinase is produced by *Bacillus cereus* using submerged fermentation (Derhab et al., 2024).

Aminoacids in plant growth and development

All amino acids contain amine group, carboxyl group and specific alkyl group in its structure. The carboxyl group of amino acid binds with amino group of another amino acid through peptide bonds to form polypeptides. They have high melting point and readily soluble in water and alcohol. Free amino acids can penetrate easier into plants compared to peptides and proteins (Abd EL Hafez *et al.*, 2011). They are nitrogen donors in the synthesis of nucleotides, chlorophyll, proteins, vitamins, carbohydrates, secondary metabolites and hormones in plants (AL-Modhaferet *et al.*, 2009). They can be applied as foliar sprays or through drip irrigation which is directly absorbed through leaves, stem and roots. Amino acids are chelated with minerals like zinc, iron, copper, magnesium, calcium and manganese to get higher mineral translocation and absorption efficiency within plants (Jacob *et al.*, 2024).

Foliar spray of amino acids enhances the growth of root and the shoot in plants (Kandi *et al.*, 2016). The water absorption capacity and the number of microorganisms present in the soil increases. The effectivity of amino acids in the plant development depends on the growth stage it is applied. It enhances plant pigments, protein content and division of cells (Ahmed and Abd El-Hameed *et al.*, 2003). Amino acids like threonine, cysteine, serine, tyrosine, and aspartic acid increases the disease resistance of plants. The tolerance of plants towards biotic and abiotic stress like heat, drought, frost and salinity is provided by proline and arginine. It is reported that foliar spray of free amino acids in wheat plants increases the photosynthetic efficiency through chlorophyll formation and protein synthesis (Kandi *et al.*, 2016). It enhances the production of plant growth regulating hormones like IAA, gibberellic acid and ethylene (Ahmed *et al.*, 2007 and 2014, Madian and Refaa *et al.*, 2011). Agricultural wastes, poultry feather waste (Bhari *et al.*, 2021), animal skin, algae, single-cell protein, whey, leather waste (Bhari *et al.*, 2021) and so on are the rich sources of amino acids.

Applications of chicken feather

Chicken feathers are used in Decoratives, fertilisers, dusters (Poopathi and Abidhaet *et al.*, 2007), bedding material (Bonser and Dawson *et al.*, 1999), feather meal, automobile and airplane industries (Tsfaye *et al.*, 2017). It is employed in many industries for packaging

material, biodegradable plastic material, biofilms, paper making, biogas (Tsfaye *et al.*, 2017) and biohydrogen production, pharmaceuticals (Moore *et al.*, 1989), biomedical sciences, waste water purification, enzyme production, cosmetics and making of electrical components.

The usage of commercial additives like peroxides, sodium chlorite, sodium nitrate, hypochlorite, formic acids, surfactants have negative effects like toxic fume production, consumption of high energy and time to remove stains, fiber damage and fiber strength reduction (Paul *et al.*, 2014). The supplementation of keratinase to the detergents as a additive is used to remove protein stains from the cloth (Zhang *et al.*, 2022). Enzymatic laundry washing gives perfect finishing to the textiles.

The usage of toxic chemicals like lime, sulfide and amines in tanneries have unpleasant effects (Akhter *et al.*, 2020) and decreased leather quality led us to look for sustainable alternative method. The keratinase can be used in enzymatic dehairing method to remove hairs from the skin without damaging the collagen by breaking disulfide bonds and so on (Arasu and Al-Dhabi, 2024).

Materials and methods:

Collection of Samples

Chicken feather samples of broiler chicken were collected from nearby slaughter house at Vallam, Chengalpattu district, Tamil Nadu. This study required *Bacillus cereus* culture which was purchased from Microbial Type Culture Collection and Gene Bank (MTCC). The seed samples were collected from agro agency at Chengalpattu, Tamil Nadu. Rice bran sample was collected from nearby rice mill at Thiruvadisoolam, Chengalpattu district, Tamil Nadu.

Preparation of keratin substrate

The feather sample collected from slaughter house was properly washed with tap water for 3-4 times to remove blood residues and unwanted impurities. Then it was washed with detergent and final washing was carried out using distilled water. It was dried at room temperature for 24 hrs to remove the moisture from feathers. Then dried chicken feathers were stored in the air tight container at room temperature for further use.

Nutrient broth preparation

Nutrient broth was prepared by adding 1.3 g of commercially available nutrient medium in 250ml Erlenmeyer flask. Then 100 ml of distilled water was added to the flask and the solution was swirled gently to dissolve the components. Before autoclaving, the pH of the medium was adjusted in the range of 7.0 ± 0.2 . The medium could be heated until clear solution was obtained to achieve complete dissolution. Then media was sterilized by autoclaving at 121°C , 15 lbs pressure for 15 minutes. After cooled down, the pH of the medium was adjusted between 7.0 ± 0.2 .

Inoculation of bacteria

The bacteria were cultured on the nutrient broth. The nutrient broth had peptone, beef extract and sodium chloride. It was prepared by dissolving 3.25 g in 250 ml distilled water at pH 7.0 and sterilised by autoclaving at 121°C , 15 lbs for 15 minutes. Meanwhile, laminar air flow hood was sterilised. It involves surface sterilisation with 70% ethanol along with air flow. After that UV was switched on for 15-20 minutes to avoid contamination of culture. Then nutrient broth was placed in the laminar air flow hood. The Bunsen burner was ignited. The loop was sterilised with 70% ethanol. Then the loop was heated until it becomes red hot in colour. After cooled down, a loopful of culture was taken and inoculated in the nutrient broth. Then the inoculated flask was placed in shaker incubator with 37°C for 24 hrs at 150 rpm.



Fig. 3: Sterilized nutrient broth.

Methods of screening

Primary Screening

The proteolysis of casein by *Bacillus cereus* was observed through primary screening. The chicken

feathers were cut into small pieces roughly in the length of 1 cm with a pair of scissors. Then 0.5 g of chicken feathers were added to 50 ml of basal medium in two different 250 ml Erlenmeyer flask. Both the flasks were sterilised by autoclaving at 121°C , 15 lbs pressure for 15 minutes. The pH of the sterilised basal media was adjusted to 7.0.

Screening of feather degradation

The sterilised basal medium flask was inoculated with 1ml of 24hrs old bacterial culture and the uninoculated flask was considered as control. Both the flasks were incubated in shaker incubator at 80-100 rpm in the temperature of 37°C for 72 hrs. After 72 hrs, the feather degradation was observed visually by compared with that of control. Then chicken feathers were filtered and washed individually from each flask. The washed feathers were dried.

Solid-state fermentation of feathers

The keratinolytic microorganism such as *Bacillus cereus* was cultured in a medium containing chicken feather and rice bran as a carbon and nitrogen source supplemented with trace salts. To prepare this, 200 ml of chicken feather media was added in two different 500 ml Erlenmeyer flask. Both the flasks were sterilised in autoclave at 121°C , 15 lbs pressure for 15 minutes. After autoclaving the pH of the media is adjusted to 7.0. After cooled down, 8 ml of 24 hrs old bacterial was inoculated and the uninoculated flask was considered as control. Both the flasks were placed in shaker incubator at 200 rpm at 37°C for 72 hrs.

Recovery of feather hydrolysate

After 72 hrs of incubation, the feather degradation was observed visually by comparing with that of control. Then the fermentation medium was filtered and the filtrate is referred as feather hydrolysate. The obtained chicken feather residues were washed with distilled water for 2-3 times to remove the unwanted particles. These feathers were dried at room temperature for 24 hrs and used for detection of feather degradation percentage.

Determination of feather degradation Percentage

The feather degradation percentage was calculated from dried residual feathers using the below mentioned

formula.

Percentage of degradation = $(TF - RF) \times 100 / TF$
Where, TF = Total weight of feathers was added to the medium

RF = Weight of residual feathers

Determination of aminoacids

The feather hydrolysate was centrifuged at 4000 rpm for 10 minutes. The clear supernatant was recovered analysed in Reverse Phase High Performance Liquid chromatography (Agilent 1100 HP-HPLC) to detect the types and quantity of amino acids present in the feather hydrolysate. The column was made of C18 molecule and the HPLC system was purged to remove impurities. 10 μ L of the feather hydrolysate supernatant, 60 μ L borate buffer and 10 μ L of Orthophthalaldehyde reagent was added in dilution vial. This mixture was mixed well in cyclomixer. 50 μ L of this mixture was injected into HPLC system using Hamilton syringes. The chromatogram and area of the peaks were obtained using Lab Solutions software after running the sample.

Detection of Indole acetic acid in hydrolysate

The obtained feather hydrolysate was centrifuged at 4000 rpm for 10 minutes. The clear supernatant of the feather hydrolysate was analyzed for the presence of IAA. The tryptophan act as an inducer for IAA synthesis by the bacteria through various interrelated metabolic pathways. The presence of IAA was indicated by Salkowski reagent. This reagent was prepared by dissolving 1ml of 0.5 M FeCl_3 solution in 49 ml of 35% sulphuric acid or perchloric acid. Estimation of IAA was done by adding 1 ml of Salkowski reagent to 0.5 ml of hydrolysate supernatant and observed in the colour change.

Effect of feather hydrolysate on plant growth

The feather hydrolysate is the source of soluble proteins, peptides and free amino acids.

Sowing of seeds

The application of feather hydrolysate in the seed germination and growth of plants was studied on three different plants such as *Vigna radiata*, *Abelmoschus esculentus* and *Oryza sativa*. Seeds were surface sterilized with 0.01% mercuric chloride (HgCl_2) solution for 5 min by shaking and then washed with

distilled water. This study was carried out by filling 1 kg of sand soil in six different plastic cups. Two cups were used for sowing each type of seed sample and 30 seed samples of *Vigna radiata*, *Abelmoschus esculentus* and 40 seed samples of *Oryza sativa* were sown cup in each cup individually.



Fig. 4: Soil preparation for sowing seeds.

Study of feather hydrolysate as biofertilizer

The feather hydrolysate was diluted with water in the ratio of 1:4. In a day, the sample group was irrigated with 60 ml of feather hydrolysate and the same quantity of water was supplied to the control group. The hydrolysate and water were provided as foliar spray. These cups were incubated for 10 days at room temperature. Then, the seedlings in sample group and control group were observed for number of seeds germinated, length of shoot and length of roots. The percentage of seed germination was calculated as follows,

$$\% \text{ seed germination} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds planted}} \times 100$$

Sample collection

The feather sample was collected from slaughter house, seed samples were collected from nearby agro agency and rice bran was collected from rice mill. All these samples were collected from Chengalpattu, Tamil Nadu



Fig. 5: Collection of broiler chicken feather.

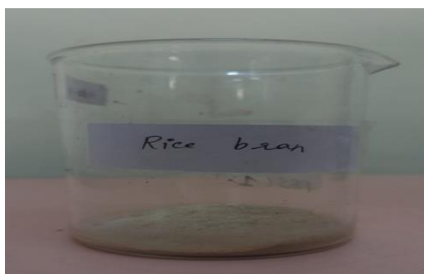


Fig. 6: Collection of rice bran.

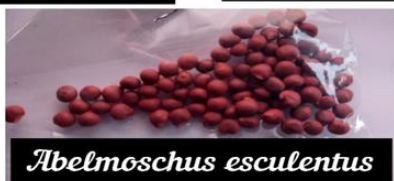


Fig. 7: Collection of seeds.

Chicken feather preparation

Raw chicken feathers were washed with water and detergent to remove blood stains and impurities. It was dried at room temperature for 24 hrs which retains fluffiness nature of white chicken feathers.



Fig. 8: Pretreated chicken feathers.

Primary Screening of Casein proteolysis by *Bacillus cereus*

A clear zone of hydrolysis was formed on the skimmed milk agar plate after 24 hrs of incubation at 37°C. The clearness of 20 mm was observed in the media. This

zone formation shows the production of extracellular protease enzyme by *Bacillus cereus* which degrades casein in the media.



Fig. 9: Primary screening for keratinase production.

Secondary screening of keratin hydrolysis by *Bacillus cereus*

The sample which was incubated with bacteria at 37°C for 72 hrs was observed for colour change. The media colour was changed from white to powdery yellow and the chicken feather structure was degraded. This confirmed that *Bacillus cereus* produces keratinase enzyme which in turn degrades keratins of chicken feather. The feather samples in control were not degraded.

Feather hydrolysate production by Solid-state fermentation

After 72 hrs of incubation, visual examination of the flasks revealed that colour of sample media was turned into yellow with powder consistency. The pH of the sample was shifted from neutral to alkaline. The feathers in sample media were not easily recognisable which indicates that the feathers were degraded. The feather hydrolysate was recovered through filtration. The residues were washed with distilled water and dried at room temperature for 24 hrs to obtain undegraded feathers for calculation of degradation percentage. It is found that 93% of feathers were degraded by *Bacillus cereus*.

Detection of indole acetic acid

Addition of 2 ml Salkowski reagent to 1 ml of supernatant of centrifuged feather hydrolysate develops pink colour which indicated the presence of IAA.

Amino acids of feather hydrolysate

The analysis of feather hydrolysate in RP-HPLC revealed that 18 types of amino acids were present in which L-Lysine concentration was dominating. The concentration and the types of amino acid are listed below.

Result of feather hydrolysate as biofertilizer

The effect of feather hydrolysate on plant growth was studied on three different plants such as *Vigna radiata*, *Abelmoschus esculentus* and *Oryza sativa*. The seeds irrigated with feather hydrolysate was considered as sample whereas seeds supplied with water was considered as control.

Study of seed germination

The seed germination percentage was higher in sample when compared with that of control.

Table 4. Seed germination percentage of samples

Name of plant	control	sample
<i>Vigna radiata</i>	60%	86.6%
<i>Abelmoschus esculentus</i>	40%	52%
<i>Oryza sativa</i>	85%	92.5%

The duration of seed germination in sample and control was compared. This revealed that the germination was initiated a day earlier in *Vigna radiata* and two days earlier in *Abelmoschus esculentus* and *Oryza sativa* in sample.

Effect of feather hydrolysate in the development of shoot

The seed samples were incubated for 10 days and the length of shoot was measured in both samples and control for each day. The observed rate of shoot development in sample than control of all plants is given below.

The comparison of shoot development in sample and control of *Vigna radiata*, *Abelmoschus esculentus* and *Oryza sativa* clearly indicates that shoot length of sample plants was higher than control plants. The feather hydrolysate helps to increase the growth of plants.

Effect of feather hydrolysate in the growth of root

The analysis of root development was done by taking a seedling from sample and control of *Vigna radiata*, *Abelmoschus esculentus* and *Oryza sativa*. Then the differences in root length between control and sample was analysed. The obtained result shows that roots of sample plants are well developed than control plants which indicates the potential of hydrolysate as fertiliser on plants.

Conclusions

The present study showed the eco-friendly degradation of feather by microbes and agricultural wastes for safer poultry waste disposal and production of amino acids from a cheap raw material. This research proves to be a useful tool for recycling feathers into valuable products, environmentally safer disposal method and its various applications in textile, cosmetics, pharmaceuticals and leather industries.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

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