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Screening and Characterization of Feather Degrading Bacteria from Poultry Waste and it's Potential of Enzyme Activity

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A B S T R A C T

The present study deals with isolation and characterization of feather degrading bacteria. A feather degradation bacterial strain was isolated from soil where poultry feathers were dumped by the poultry farms as a waste product. Sixteen native strains were isolated and their morphological, cultural and biochemical characteristics were completely studied according to Bergey's Manual of bacteriology. These novel strains could be a potential candidate for degradation and utilization of feather keratin by screening the protease and keratinase enzyme activity. This bacterial strain was analyzed further studies and therefore promising strains for the management of feather waste through biotechnological processes.

***Key words*:** Feather, Keratin, Feather degrading bacterium, Poultry waste

Feather wastes are generated in bulk quantities as a by-product of commercial poultry processing. Feathers represents 5-7% of the total weight of the mature chickens. Typically, as each bird has up to 125gms of feather, the weekly worldwide production of feather waste is about 3000 tonnes. As a result, poultry processing plant produce a substantial amount of feathers as waste by-Product and represent a sizable waste disposal problem. 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. 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]. Disposal of this bulk waste is a global environmental problem accounting to pollution of land and underground water resources. Feather constitutes over 90% protein, main component being beta-keratin, a fibrous and insoluble protein. Keratins are the most abundant proteins in epithelial cells of vertebrates and represent the major constituents of skin and its appendages such as nail, hair, feather and wool. The degradation of keratinous material has medical and agricultural importance [2-3]. A group of proteolytic enzymes that are able to hydrolyze insoluble keratins more efficiently than other proteases are called keratinases. Bacterial keratinases are of particular interest because of their action on insoluble keratin substrates and generally on a broad range of protein substrates. Keratinase is an extracellular enzyme used for biodegradation has the ability to degrade feather waste. Microbial inoculums might convert waste feathers into supplement for digestive feed for poultry, livestock and fish and it also used for production of biogas. It is cost effective and ecofriendly. In the present investigation, the keratin utilizing bacteria were isolated from the feather dumped soil. Isolation of bacteria based on its morphology and identification based on the cultural characteristics. Screening of potential bacteria from protease and keratinase enzyme activity.

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MATERIALS AND METHODS

Soil samples were collected from the two sites of poultry farm of Thanjavur district were taken either deeper than 10cm. Decaying feathers were present into the soil sample. The samples were collected in polythene bag marking as AI and AII. Soil sample was aged and greyed with decayed feathers was incubated and kept for monitoring quality improvement. Soil samples were brought the Laboratory. Soil samples was weighed and kept in the closed chamber in the moist atmosphere at room temperature within 48 hrs.

Isolation of feather degrading bacteria

One gram of feather dumped soil sample transferred in 9 ml of sterile distilled water. The samples were serially diluted 10^{-6} and six fold dilutions were plated on nutrient agar and incubated for 24 hours at 37°C. The different colony morphology were picked and purified using streak plate method.

Culture characteristics observation

Bacterial cells were transferred from preserved culture with fresh nutrient agar plate. After overnight incubation growths from the plates were again transferred with second set of nutrient agar plate incubated and observed the actual growth patterns on culture plate were performed. Control was maintained. Morphological characteristics and biochemical characterization were tested by Bergey's Manual method. Morphological characteristics of isolated bacterial stains have observed with the following features of the colonies are size, whole colony morphology, margin or edge, elevation, pigmentation, optical features, textures of the surface were performed with respective organisms.

Biochemical test

Biochemical test was established the enzymatic capabilities of a given bacterial isolates as well as the isolates ability to grow or survive the presence of certain inhabitants (salts, surfactants, antibiotics).

Gram staining method

Gram positive bacteria have cell walls that contain thick layers of peptidoglycon. These strains are purple. Gram negative bacteria have thin layer of peptidiglycon so they are in pink in colour. During this method, applying primary stain (crystal violet) to a heat fixed smear, followed by mordant (gram iodine), rapid decolourization with alcohol and lastly counterstaining with safranin.

Indole test

Indole is the product of the breakdown of another amino acid tryptophan by the enzyme tryptophanase. To test for indole; Kovacs reagent was added with SIM medium following growth. If indole is present a red ring were indicated around the test tube surface.

Methyl red and voges proskaure test

(MR-VP TEST) was used to determine the ability of a bacterium to oxidize glucose and produce stable acid end products. Methyl red is a pH indicator (red at pH less than 4.4 and yellow at a PH greater than 6).MR-VP broth was used. Acid production is positive for methyl red and end product of neutral pH is positive for voges proskaure test.

Citrate utilization test

Simmon`citrate agar utilize sodium citrate as its sole carbon source. Bromothymol blue was included as a pH indicator; the medium initially is green. Organisms capable of using citrate as a carbon source turn the medium into Prussian blue.

Catalase test

When hydrogen peroxidises was added to a colony of catalase producing bacteria, it was broken down and the oxygen that was produced can be seen as bubbles.

Carbohydrate fermentation test

To determine the ability of bacteria with fermented carbohydrates with the production of an acid/gas. If the medium changes from colourless to yellow and gas bubbles and found in Durham's tube then it indicated acid and gas production. If no change observed in the colour of medium then sugar was not degraded by bacteria.

Oxidase test: To determine the ability of microbes to produce cytochrome oxidase enzyme. Note the purple to dark purple colour after the colonies have been added to filtered paper moistened with oxidase reagent was positive.

Triple sugar iron test

TSI was a differential medium that contains lactose, sucrose, a small amount of glucose, ferrous sulphate and PH indicator phenol red. It was used to differentiate enteric based on the ability to reduce Sulphur and ferment carbohydrates. Red slant /Red butt=no fermentation, Red slant/yellow only glucose fermentation, yellow slant/yellow butt=lactose and sucrose fermentation, dark colour hydrogen sulphide produced. It also differentiated between groups capable of reducing Sulphur to hydrogen Sulphide.

Screening of protease and keratinase from potential bacterial sp

All The bacterial strains were produced protease enzyme in LB broth containing growth shaken flask and determined quantity at 420nm by spectrophotometer. The proteolytic activity of enzyme was determined using the procedure described earlier [4]. Similarly, screenings of potential bacteria from keratinase enzyme activity in basal medium of feather meal broth were screened at 440nm in spectrophotometer. Basal salt medium of pH 7.5 containing gl^{-1}; NH_4Cl^{-1}, $NaCl^{-1}$, $K_2HPO_4^{-6}$, $KH_2PO_4^{-8}$, $MgCl_2^{-2}$, and yeast $extract^{-1}$. Crude cultured broth was assayed for Protease and Keartinase activity were measured in first, fifth, tenth and fifteenth day of incubation respectively.

RESULTS AND DISCUSSION

Preliminary identification of bacteria was done by serial dilution method shown in (Table 1, Plate 1). By using nutrient agar medium and serial dilution method ,microbiological study were performed. Microbial colonies were enumerated as Colony Forming Unit (CFU). The bacterial colonies with different morphology were picked and purified using streak plate method. Different colonies were noted on nutrient agar medium. However, sixteen colonies growing well on this medium were chosen for further studies, therefore series of bacterial strains were isolated from the waste dump having feathers.

Table 1 Isolation of bacteria from feather degraded soil sample

Dilution factors	Sample AI	Sample AII
	Number of colonies (CFU/ml)	
10^{-4}	TNTC	TLTC
10^{-5}	TLTC	TLTC
10^{-6}	TLTC	TNTC

TNTC- Too numerous to count
TLTC- Too low to count

Studies have showed that many microorganisms are isolated from poultry feather waste. These microorganisms include bacteria, actinomycets and fungi [5-6]. Diverse group of microorganism are reported to isolated from feather waste like fungi., *Aside sp., Stachybotrys alba*, etc.), actinomycetes (*Streptomyces pactum, S. Alvs, s. Thermoviola ceus, S.* (*Doratomyces microsporus, Alternaria radicina, Trichurus spiralis, Aspergillus sp., Rhizomucor sp Fradiae, Thermoactinomyces candidus* etc.), and several bacterial species (*Fervidobacterium islandicum, Pseudomonas aeruginosa, Microbacterium sp*., and many species of *Bacillus* including *Bacillus licheniformis* and *B. puilus*) earlier. Among

bacteria, *Bacillus spp*. appears to be promising for keratinase production on commercial scale.

Sixteen selected colonies that grew well on this medium were selected to be sub cultured at frequent intervals until well adapted and purified colonies were obtained. Initial morphological identification of isolate was done by gram staining. Morphological characteristics of isolated strains such as growth, shape, surface, margin, colour, elevation, consistency, opacity were noted for the nine selected strains. We propose MIRR as the strain designation to indicate the isolation of the novel strain from poultry waste. All the isolate showed good growths with varying level of morphological characteristics are summarized in (Table 2). Totally 16 bacterial species were identified such as *Bacillus subtilis, Bacillus sp., Brevibacillus parabrevis, Brevibacillus brevis, Escherichia coli, Clostridium butyricum, Flavobacterium odoratum, Morococcus cerebrosus, Oscillospira guilliermondii, Pseudomonas aerogenosa, Pseudomonas fluorescence, Proteus vulgaris, Proteus sp., Serratia fonticola, Staphylococcus aureus* and *Streptococcus pneumoniae*.

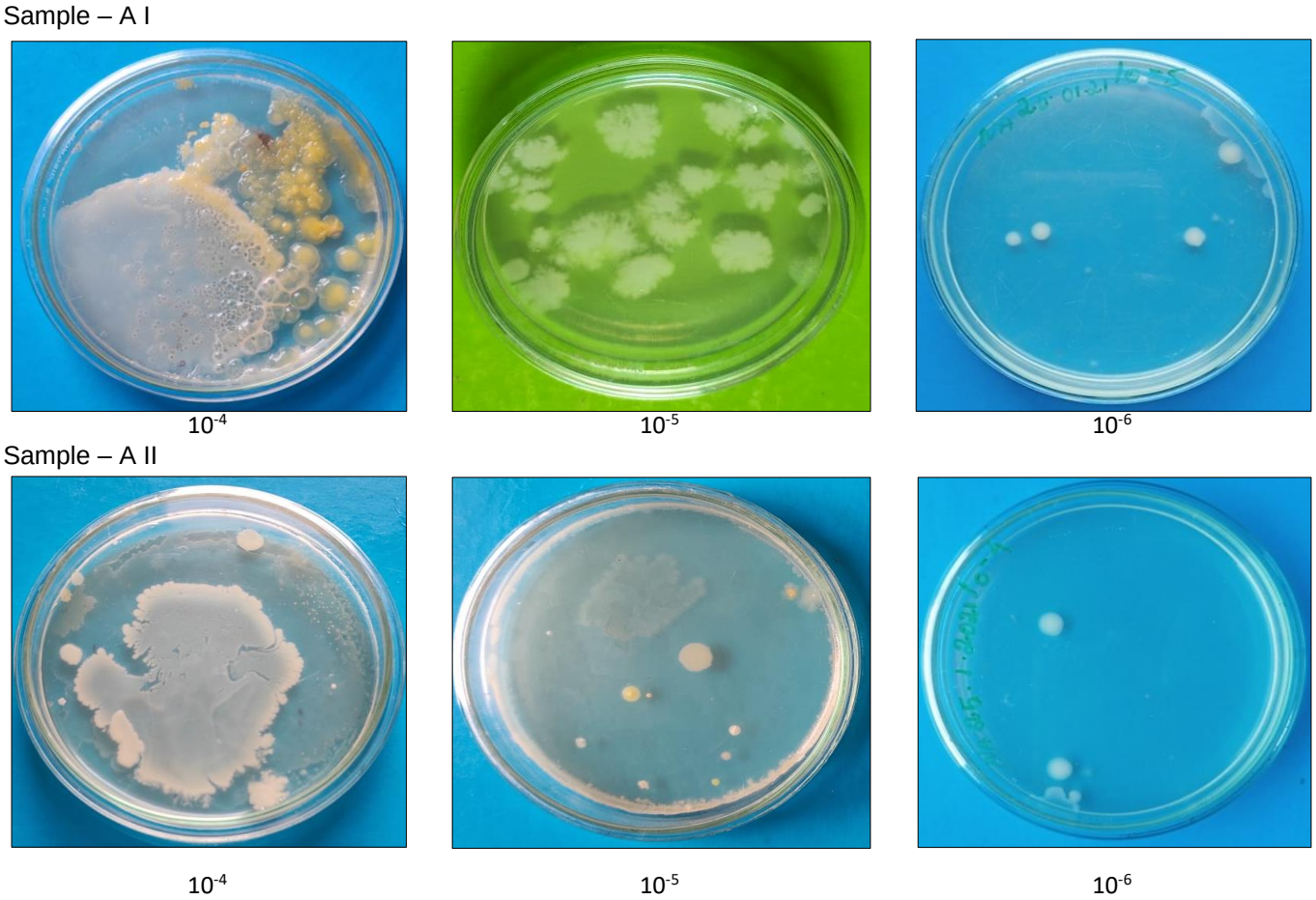

Plate 1 Isolation of bacteria from feather degraded soil sample

Table 2 Morphological characteristics of isolated bacteria

Strains	Morphological characters							
	Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
MIRR 1	Rapid	Circular	Rough	Irregular	White	Convex	Viscous	Opaque
MIRR 2	Rapid	Circular	Rough	Entire	Creamy white	Raised	Viscous	Opaque
MIRR 3	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
MIRR 4	Rapid	Rhizoid	Smooth	Lobate	Milky white	Flat	Viscous	Opaque
MIRR 5	Slow	Punctiform	Rough	Irregular	White	Umbonate	Adhesive	Opaque
MIRR 6	Slow	Irregular	Rough	Irregular	Milky white	Raised	Buttery	Opaque
MIRR 7	Rapid	Rhizoid	Smooth	Erose	Milky white	Pulvinate	Buttery	Opaque
MIRR 8	Rapid	Punctiform	Rough	Undulate	White	Pulvinate	Adhesive	Opaque
MIRR 9	Rapid	Punctiform	Rough	Erose	White	Convex	Viscous	Opaque
MIRR 10	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
MIRR 11	Slow	Circular	Smooth shiny	Entire	Light orange	Pulvinate	Buttery	Opaque
MIRR 12	Slow	Irregular	Glistening	Entire	Pale yellow	Pulvinate	Buttery	Translucent
MIRR 13	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
MIRR 14	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
MIRR 15	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
MIRR 16	Slow	Circular	Smooth shiny	Entire	Creamy white	Pulvinate	Buttery	Opaque

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Some previous studies also supported these findings, the uses of keratin utilizing microorganisms are being studied in applied microbiology where active degraders of feather keratins is greatly needed [7]. Although the nature of keratin-rich wastes

such as feathers is resistant to degradation by common proteases, keratins are not accumulated in nature, suggesting that they are degraded by microorganisms [8]. Numerous microorganisms have been isolated from different environments that are rich in keratin and have been applied to degraded keratin-containing wastes from different resources [9-10]. Molyneaux [11] conducted an extensive classification of isolate but did not assign a species name. Many of the results of biochemical tests and conducted match those we observed for our native strains too. Different biochemical tests were done for characterizing the bacterial strains and the result are given in the (Table 3).

Table 3 Biochemical characteristics of isolated bacterial strains from feather degraded soil

Code of the bacteria	Biochemical Test											
	GS	IT	MR	VP	CU	CT	Carbohydrate fermentation			OX	TSI	Name of the bacteria
							Glucose	Mannitol	Sucrose			
MIRR 1	-	+	-	-	+	+	Invalid	+	+	+	A/A H_2S	*Oscillospira guilliermondii*
MIRR 2	-	-	+	+	+	-	-	Invalid	+	+	H_2S	*Flavobacterim odoratum*
MIRR 3	+	-	-	-	+	+	+	+	+	+	A/A H_2S	*Bacillus subtilis*
MIRR 4	-	+	-	-	+	-	+	+	+	+	A/A H_2S	*Seratia fonticola*
MIRR 5	-	+	+	+	+	+	-	-	Invalid	+	A/A H_2S	*Pseudomonas aerogenosa*
MIRR 6	-	+	-	-	-	-	+	+	+	-	A/A H_2S	*Pseudomonas fluorescence*
MIRR 7	-	+	+	+	+	+	Invalid	+	+	+	A/A H_2S	*Morococcus cerebrosus*
MIRR 8	-	+	+	-	-	+	+	Invalid	Invalid	-	A/A	*Escherichia coli*
MIRR 9	+	+	+	+	+	-	+	+	+	+	A/A H_2S	*Clostridium butyricum*
MIRR 10	-	-	-	+	-	+	-	+	-	+	H_2S	*Staphylococcus aureus*
MIRR 11	+	-	-	+	-	+	Invalid	+	+	-	A/A H_2S	*Bacillus* sp.
MIRR 12	-	-	+	-	+	+	+	+	+	-	A/A H_2S	*Proteus* sp.
MIRR 13	+	+	-	+	+	+	+	Invalid	Invalid	-	A/A H_2S	*Streptococcus pneumoniae*
MIRR 14	-	+	+	-	-	+	+	-	+	-	H_2S	*Proteus vulgaris*
MIRR 15	+	-	-	-	+	+	+	-	+	-	A/A	*Brevibacillus brevis*
MIRR 16	+	-	-	-	+	+	Invalid	-	+	-	A/A	*Brevibacillus parabrevis*

GS-Gram staining, IT- Indole test, MR-Methyl red, VP- Voges proskaure, CU- Citrate utilization, CT- Catalase, OX- Oxidase, TSI-Trible sugar iron test, A/A -Acid butt Acid slant, H_2S-H AL/A Hydrogen sulphide production, AL/A-Alkaline butt acid slant

Table 4 Screening of Protease from potential bacterial sp

Name of the bacteria	Quantity (IU/ml)			
	1st Day	5th Day	10th Day	15th Day
Control	0.09±0.00	0.12±0.02	0.13±0.07	0.15±0.03
Bacillus subtilis	0.31±0.08	0.82±0.08	0.84±0.11	0.84±0.13
Bacillus sp.	0.27±0.01	0.35±0.18	0.49±0.05	0.67±0.03
Brevibacillus parabrevis	0.37±0.03	0.89±0.11	0.93±0.24	0.97±0.16
Brevibacillus brevis	0.35±0.05	0.83±0.25	0.87±0.17	0.91±0.03
Clostridium butyricum	0.14±0.00	0.15±0.00	0.16±0.14	0.25±0.08
Escherichia coli	0.16±0.05	0.24±0.14	0.25±0.00	0.31±0.12
Flavobacterium odoratum	0.30±0.00	0.54±0.14	0.57±0.08	0.82±0.05
Morococcus cerebrosus	0.15±0.61	0.16±0.05	0.21±0.17	0.24±0.11
Oscillospira guilliermondii	0.14±0.03	0.16±0.06	0.21±0.05	0.23±0.01
Pseudomonas aerogenosa	0.29±0.06	0.45±0.21	0.50±0.12	0.73±0.17
Pseudomonas fluorescence	0.18±0.05	0.25±0.03	0.34±0.12	0.39±0.08
Proteus vulgaris	0.12±0.08	0.14±0.00	0.17±0.00	0.21±0.00
Proteus sp.	0.12±0.35	0.13±0.00	0.13±0.05	0.15±0.07
Serratia fonticola	0.26±0.05	0.28±0.08	0.35±0.08	0.45±0.08
Staphylococcus aureus	0.09±0.24	0.11±0.03	0.17±0.11	0.21±0.14
Streptococcus pneumoniae	0.10±0.00	0.11±0.08	0.13±0.13	0.14±0.11

Values are expressed in Mean ± Standard deviation

Table 5 Screening of Keratinase from potential bacterial sp

Name of the bacteria	Quantity (IU/ml)			
	1st Day	5th Day	10th Day	15th Day
Control	0.07±0.00	0.10±0.03	0.11±0.02	0.13±0.05
Bacillus subtilis	0.30±0.08	0.82±0.05	0.89±0.11	0.95±0.26
Bacillus sp.	0.29±0.01	0.73±0.13	0.87±0.05	0.94±0.09
Brevibacillus parabrevis	0.34±0.15	0.92±0.05	0.96±0.13	1.05±0.35
Brevibacillus brevis	0.31±0.08	0.91±0.08	0.91±0.25	1.03±0.14
Clostridium butyricum	0.17±0.04	0.36±0.11	0.41±0.14	0.43±0.17
Escherichia coli	0.18±0.05	0.48±0.03	0.51±0.17	0.57±0.05
Flavobacterium odoratum	0.26±0.04	0.75±0.00	0.86±0.18	0.91±0.23
Morococcus cerebrosus	0.14±0.00	0.43±0.05	0.48±0.11	0.51±0.04
Oscillospira guilliermondii	0.14±0.03	0.27±0.00	0.31±0.03	0.37±0.15
Pseudomonas aerogenosa	0.21±0.11	0.65±0.01	0.74±0.24	0.78±0.25

Pseudomonas fluorescence	0.23±0.06	0.68±0.11	0.79±0.07	0.83±0.18
Proteus vulgaris	0.19±0.11	0.55±0.06	0.63±0.11	0.68±0.53
Proteus sp.	0.20±0.04	0.57±0.05	0.64±0.36	0.68±0.24
Serratia fonticola	0.22±0.09	0.68±0.02	0.81±0.13	0.87±0.29
Staphylococcus aureus	0.11±0.02	0.27±0.03	0.32±0.07	0.38±0.15
Streptococcus pneumoniae	0.12±0.07	0.26±0.00	0.29±018	0.35±0.19

All the isolates showed good growth with varying level of keratinase and protease activities (Table 3-4). Screening of potential bacteria within the growth of 15th day protease activity. Among bacterial isolates, *Brevibacillus parabrevis* and Brevibacillus brevis exhibited the highest keratinolytic and proteolytic activity and efficiency to hydrolyse raw feather material. One unit of enzyme activity was the amount of enzyme that causes a change of absorbance of 0.01 under the conditions herein described. The maximum quantity protease produced bacteria was screened for further studies such as *Brevibacillus parabrevis* (0.97±0.16IU/ml) and Brevibacillus brevis (0.91±0.03IU/ml).

In this present investigation of screening of potential bacteria from keratinase enzyme activity in basal medium of feather meal broth were screened *Brevibacillus parabrevis* (1.05±0.35IU/ml) and Brevibacillus brevis (1.03±0.14IU/ml) at 440nm in spectrophotometer. Bacillus species were the predominant isolates encountered in this study; other investigators had also reported the keratinolytic activities of several species of Bacillus [12-15]. All the bacterial isolates encountered demonstrated both proteolytic and keratinolytic activities. This is similar to the observation of Daroit *et al*. [16] who reported same for *Bacillus spp*. P45. So also, *Serratia marcescens* has keratinolytic potential and protease properties capable of degrading feather meal and producing high amounts of soluble proteins and forming thiol groups [17].

CONCLUSION

Based on accumulated studies, feathers from poultry industry can be treated in an economic and environmentally friendly way by isolated strains and serve as a valuable source for other application. In the present investigation, soil sample from the poultry waste were serially diluted and sixteen well defined colonies were selected and were pure cultured. The isolates were subjected to morphological, microscopical; gram reactions and biochemical test growth at 37ºC. These bacterial potential strains were screened for the degradation of keratin in feather waste by protease and keratinase activity. This study provides knowledge on microorganism present in poultry waste. From the above discussion, these isolates possessing potential biotechnological use might be employed to remove environmental pollutant like feather present in poultry farms.

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