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Biodegradation of Chicken Feathers by Isolate *Pseudomonas* from Road Side Waste Dumping Site at Aruppukottai Town

S.Nithya and B.Govindarajan*

Department of Microbiology, V.H.N.Senthikumara Nadar College (Autonomous), Virudhunagar, India

*Corresponding author email id: bgrmphilbed@gmail.com

Abstract

Chicken is one of the popular protein sources for majority of the population. A Million tones of chicken feathers are generated every year as by product in the world wide poultry industries. Feathers are commonly disposed by land filing which causes environmental pollution and local trouble such as bad odour, etc. Traditional ways to degrade feathers such as alkali hydrolysis and steam pressure cooking but these techniques consume huge amounts of energy and time. There is an urgent requiring for new innovations enabling ecological and economical valorization of this chicken feather waste. Hence the present investigation exhibited "Biodegradation of chicken feathers by isolate *Pseudomonas* from road side dumping site at Aruppukottai town'' has been carried out.

Keywords: Biodegradation, Chicken feathers, *Pseudomonas*, Aruppukottai

Introduction

The term municipal solid waste refers to the solid wastes from residential, commercial, industrial, institutional and slaughterhouse waste. Chicken is one of the majority accepted protein sources for majority of the population. Indian Poultry Industry is emerging as the world's second largest market and growing at a phenomenal rate of 12-15% every year. India generates 350 million tones of feather per year (Agrahari *et al*., 2010). Feathers are generally disposed by land filing which causes ecological pollution and local disturbance such as odour, files and rodents. Also causes various human aliments including chlorosis, mycoplasmosis and fowl cholera (Gerber *et al*., 2007).

Feather is pure keratin protein and is unsolvable and hard to degrade due to highly rigid structure rendered by extensive disulphide bond and cross-linkages. Chicken feathers constitute 10% of total chicken weight and contain approximately 91% protein (keratin), 1% lipids and 8% water (Thygarajan *et al*., 2013).

Traditional ways to degrade feathers consume large amounts of energy and time (Cai *et al*., 2008). Degradation of poultry feathers by kerationlytic proteases offers an alternative method for efficient bioconversion, nutrional enhancement and environmental friendliness (Xu *et al*., 2009). As microbial methods are considered as cost effective and environment friendly, an interesting alternative to these techniques is microbial degradation, due to the low cost, mild process conditions, lack of the ecological hazard (Vasileva-Tonkova *et al*., 2009).

Pseudomonas is a Gram negative, rod shaped bacteria. It is a facultative anaerobic, motile organism. These are commonly present in soil, compost and water. Pseudomonas produces the Keratinase enzyme and degrades the chicken feathers (Minghai Han *et al*., 2012).

Microorganisms have been reported to produce Keratinase in the presence of keratin substrate. Keratinase producing microorganisms have ability to degrade chicken feathers (Cai *et al*., 2008). Bacterial strains are known which are capable of degrading feathers. These bacterial strains produce enzymes which selectively degrade the bête keratin present in feathers. These enzymes make it possible for the bacteria to obtain carbon, sulfur and energy for their growth and maintenance from the degradation of beta keratin. An enzyme capable of degrading protein is known as a protease and is described as having proteolytic activity. An enzyme which degrades keratins can also be described as having keratinolytic activity (Suntornsuk *et al*., 2013).

There is an urgent need for new processes enabling ecological and economical valorization of this resistant chicken feather waste. Hence the present investigation exhibited "Biodegradation of chicken feathers by isolate *Pseudomonas* from road side dumping site at Aruppukottai town'' has been carried out.

Materials and Methods

Soil Sample Collection

Soil sample collection site locations were fixed previously. Sampling location was cleaned properly by scrapping the litter and plant parts from the surface. Two samples of soils were

collected in Aruppukottai, Virudhunagar district, Tamilnadu, India. All samples were coded. For soil samples the codes are given as S1 to S2.

Collection of soil

Soil samples were collected from the top 15 cm layer of the sampling stations. Prior to collection, top layer soils were hand sorted and plant materials as well as litter were carefully removed from the soil. About 10 g of soil were taken in individual labeled sterile container from two sites.

Preparation of soil sample

Soil samples were air dried in shade by spreading on newspapers. They were cleaned by discarding plant residues, gravels, coarse materials, stones and other debris if present. The cleaned soil samples were stored in a clean sterile glass container. The soil samples were labeled immediately in the form of tags to prevent sample misidentification and given a unique sample number, sampling code, sampling time and special note if any.

Feather sample collection and preparation

Chicken feathers collected from the road side waste dumping site. After collection, stones, tissues and blood stains removed and the feathers were washed with distilled water twice and subsequently dried in hot air oven at 50ºC. After this feathers were stored for further use. The sterilized feather was taken and cut into small pieces (Vuppu Suneetha *et al*., 2011).

Isolation of *Pseudomonas*

The collected soil samples were serially diluted up to 10-9 and 0.1 ml from 10-6 dilution was spread plated on to Pseudomonas isolation agar (Appendix-1) medium. The petridishes were incubated at 37ºC for 24 hours. The individual's colonies were picked and purified using streak plate method (Samuel Pandian *et al*., 2012).

Identification of *Pseudomonas*

Isolates were identified based on cultural, morphology and biochemical characteristics and were compared with Bergey's Manual of Determinative Bacteriology.

Experimental Design

Biodegradation experiments were carried out in 250ml conical flak. Before putting into the culture media feather was weighed using electronic balance and recorded. Control without inoculate were processed under same conditions. The feather degradation was done by inoculating 1ml of each isolates into 250ml Erlenmeyer flask containing 100ml liquid medium (Appendix-3). The flask were shaken at 160rpm in Research department of Botany, VHNSNC (Autonomous) and incubated at room temperature for 12 days. After 12 days incubation the residual feathers were harvested from the fermentation media by filtering it over whatman filter paper and stored in refrigerator for further analysis. The supernatant was collected for examine protein content.

Determination of degradation

The percentage of degradation of feathers by Pseudomonas was determined by calculating the percentage of weight loss of feather. The percentage of weight loss was calculating by the formula

$$\text{Percentage of weight loss} = \frac{initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Protein Estimation

The amount of protein present was assayed by the Lowry's method (1951) using bovine serum albumin (BSA) as standard. The color developed was read at 660nm.

Result

Table 1: Different study area Aruppukottai municipality road side dumping site

Site code	Location	Use
S1	Near police station, Madurai road	Garbage dumping
S2	Near Thimmamal temple, Thiruchuli road	Garbage dumping

Table 2: Biochemical characteristics of bacteria isolated from road side dumping site of Aruppukottai

Characteristics	Result
Morphological characteristics	
Colony	Circular, smooth, moist
Color	Yellow
Shape	Rod
Physiological characteristics	
Motility	Motile
Endospore	Non sporulating
Biochemical characteristics	
Gram staining	G-
Indole	Negative
Methyl red	Negative
Voges Proskauer	Negative
Simmon citrate	Positive
Starch Hydrolysis	Negative
Gelatin liquefaction	Positive
Nitrate reduction	Positive
Oxidase	Positive
Catalase	Positive

Table 3: Degradation of feather by *Pseudomonas*

Name of the organism	Days of treatment	Feather weight (g)	Weight loss (%)
Pseudomonas	0	1	0
	6	0.62	48
	12	0.14	86

Table 4: Changes in Protein content estimated by Lowry's method

Days of treatment	Protein (mg/ml)
0	0
6	210
12	450

Fig 1: Soil sampling site 1

Fig 2: Soil sampling site 2

Fig 3: Soil sample

Fig 4: Chicken feather

Fig 5: Feather degradation by *Pseudomonas*

Discussion

In the present investigation, the bacterial strain isolated from Aruppukottai road side garbage dumping site soil was a Gram Negative rod and it was identified as *Pseudomonas* on the basis of results obtained in biochemical tests (Table 2). Negative results were obtained for Gram staining, Methyl red, Voges proskauer and Indole. Simmon citrate, Catalase and Gelatin liquefaction tests showed positive results. Similar result was observed by Minghai Han (2012) and John and Anyanwu (2016).

The biodegradation of chicken feather study was carried out by employing *Pseudomonas* as test organism. They were exposed to two different substrates viz., control and experimental for 12 days. During the treatment period, the observations were made for change in color, development of turbidity, slimy layer formation and breakage of barbules were observed and the results are shown in Table 3. After 6 days 48% of feathers' weight was completely

degraded. The 86 % of degradation was observed at 12th day during experiment. It is evident that *Pseudomonas* biodegrade the chicken feathers.

The present study clearly showed that the chicken feathers were biodegraded by *Pseudomonas*. Similar observations were made by Iva Pernicova *et al*., (2019) when they used *Pseudomonas* for biodegradation study, the *Pseudomonas* bioremediate the feather. *Pseudomonas* produces the Keratinase enzyme and degrades the chicken feathers.

This bacterium isolated from poultry waste and able to degrade feather keratin when using feather as a primary source of carbon and energy. Suharti *et al*.,(2018) produces protease to degrade various fibre proteins.

During the feather degradation more amount protein is released. In the time my research period 210mg/ml of protein released at 6th day and 450 mg/ml of protein released at 12th day. So it can be used as an animal feed, common protein sources used in animal feed are expensive but these protein sources are inexpensive. Similarly it was observed by Bishmi *et al*., 2015.

Conclusion

- ✓ The soil and chicken feather samples were collected from two different sites of road side solid waste dumping site at Aruppukottai town.
- ✓ The isolation of *Pseudomonas* were done by serially diluting the soil samples and spread on the Pseudomonas isolation agar plates.
- ✓ Based on the morphological and biochemical characteristics, the isolates were identified as Pseudomonas.
- ✓ Feather samples were washed with distilled water and dried at 50ºC in hot air oven.
- ✓ Feather meal medium was prepared in a 250 ml conical flask inoculated with Pseudomonas and one flask kept as control incubated at 160 rpm at room temperature.
- ✓ The flasks were observed for change in color, development of turbidity and breakage of barbules. The experimental setup was maintained for 12 days. After 6 days 48% of feathers' weight was completely degraded. The 86 % of degradation was observed at 12th day during experiment.
- ✓ The protein content of the hydrolytes were estimated by Lowry's method. 210mg/ml of protein released at 6th day and 450 mg/ml of protein released at 12th day.

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Appendix-1

Pseudomonas Isolation agar

Peptic digest of animal tissue	20.00 gms
Magnesium chloride	1.400 gms
Potassium chloride	10.00 gms
Triclosan (Irgasan)	0.025 gms
Agar	15.00 gms
Ph	7.0

Appendiex-2

Nutrient broth

Peptone	5.00 gms
Sodium chloride	5.00 gms
Peptone	1.5 gms
Yeast extract	1.5gms
pH	7.4

Appendix-3

Feather meal agar

Ammonium chloride	0.05 gm
Sodium chloride	0.05 gm
Dipotasssium hydrogen phosphate	0.03 gm
Potassium dihydrogen phosphate	0.04 gm
Magnisem chloride	0.024 gm
Yeast extract	0.1 gm
Feather	1 gm
pH	7.5