International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB)
Volume 1, Issue 1, June 2015, PP 25-30
www.arcjournals.org

Biodegradation of Poultry Feathers using a Novel Bacterial Isolate P*seudomonas aeruginosa*

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Abstract: *Feather waste is generated in large amounts as a by-product of commercial poultry processing. This residue is almost pure keratin, which is not easily degradable by common proteolytic enzymes. Pseudomonas aeruginosa, a novel, raw chicken feather degrading bacterium, previously isolated and identified by morphological and biochemical tests in our laboratory, was used in the present study. The organism was inoculated along with 0.2, 0.4, 0.6, 0.8 and 1g of feather and incubated for five days. The degrading capacity was observed by changes in pH, protein and carbohydrate content and biomass. These data indicate that Pseudomonas aeruginosa could be useful in the management of poultry wastes.*

Keywords: *Feather waste, Keratin, Pseudomonas aeruginosa, pH, protein, carbohydrate and biomass*

1. INTRODUCTION

Feathers constitute the major bulk of biological waste generated by local butchers and poultry processing industries in India [1]. According to a recent report of USDA, annual consumption of poultry in India was 2.3million tons in 2010 and it is expected to increase at a tremendous rate. Due to poor management of waste, the by-products of poultry industry (especially feathers) have become one of the major pollutants due to their recalcitrant nature [1, 2]. About 90% of feathers consist of keratin, which is a fibrous and insoluble structural protein consisting of β-helical coils joined together by disulfide linkages [3].This structural feature enables it to resist adverse environmental conditions and degradation by proteases [4]. Therefore, feathers are considered as a biological waste and cause serious environmental problems. However, feathers are considered as a good source of essential amino acids [5].

Microorganisms which produce keratinolytic protease may have important use in hydrolysis of keratin-containing wastes from leather and poultry industries. They represent an attractive alternative method for efficient bioconversion and improving the nutritional value of keratin wastes by developing economically and environmentally friendly technology [6, 7]. The potential applications of such microbial keratinases have been recently reported [6, 7, 8, 9].

The Keratinase producing bacteria like *Bacillus subtilis, B. cereus, B. amyloloquefaciens* and *B. megaterium* and fungal species of *Aspergillus, Penicillum, Cephalosporium, Neurospora* and *Rhizopus* are major keratinase producing microorganisms. Microorganisms such as fungi, bacteria, actinomycetes and algae are effectively producing keratinase[10].Keratinases have applications in traditional industrial sectors including feed, detergent, medicine, cosmetics and leather manufacturing [11]. In the present study, an attempt has been made for testing the efficiency of the strain *Pseudomonas aeruginosa* in feather degradation.

2. MATERIALS AND METHODS

2.1. Collection of samples

Soil samples were collected from various poultry processing regions at Madurai in sterile containers and transported to laboratory immediately for analysis.

2.2. Isolation of feather degrading bacteria

The collected soil samples were serially diluted up to 10^{-9} and 0.1 ml from 10^{-6} dilution was spread plated on to nutrient agar medium [12]. The petridishes were incubated at 37^0C for 24 hours. From the growth of colonies, one colony was selected.

2.3. Identification of bacterial strain

The resistant bacterial strain was selected and identified after conducting biochemical testes as *Pseudomonas aeruginosa* by adopting Bergey's Manual [13].

2.4. Feather Degradation

Chicken feather in different quantities (0.2, 0.4, 0.6, 0.8 and 1g) was transferred to 100ml of minimal medium (Dextrose 1g, Ammonium sulphate 1g, Dipotassium phosphate 7g, Monopotassium phosphate 2g, Sodium citrate 0.5g and Magnesium sulphate 0.1g). 0.1ml of *Pseudomonas aeruginosa*from the logarithmic phase of the pure culture maintained in nutrient broth was inoculated and incubated for five days in a shaker at 120 rpm. Every day samples were taken from culture medium for measuring pH, protein content, carbohydrate content and biomass. The amount of protein in culture medium was quantified by the method explained by Lowry *et al* [14]. The amount of carbohydrate in culture filtrate was quantified by Anthrone method [15]. The biomass was estimated after centrifugation. Ten ml of culture medium was centrifuged at 10,000 rpm for ten minutes. Then the supernatant was removed and the pellets were dried. The weight was measured and the biomass was calculated as gram per litre.

2.5. Statistical analysis

Two way analysis of variance (ANOVA) was carried out for pH, protein, carbohydrate content and biomass for the variables, treatment period and feather quantity using MS Excel. Variability was considered only when the calculated F value was greater than the tabulated F value at P is less than or equal to 0.05 [16].

3. RESULTS AND DISCUSSION

The bacterial strain isolated from the soil was a Gram negative rod and it was identified as *Pseudomonas aeruginosa* on the basis of results obtained in biochemical tests (Table 1). Negative results were obtained for Gram staining, Methyl Red, Voges Proskauer andIndole.Simmons Citrate, Catalase and Gelatin Liquefaction tests showed positive results. Keratin degradation is mostly performed by Gram-positive bacteria [6], although there are a few reports on feather-degrading Gram-negative bacteria [7, 17].

Table 1. *Biochemical tests used for the identification of isolated organism*

S.No	Biochemical Tests	*Pseudomonas aeruginosa*
1.	Colony character	Smooth Wrinkled
2.	Colony size	Medium
3.	Cell type	Rod
4.	Gram reaction	-
5.	MR test	-
6.	VP test	-
7.	Indole test	-
8.	Catalase test	+
9.	Citrate test	+
10.	Gelatin Liquefaction	+
11.	Cellobiose	-
12.	Lactose	-
13.	Maltose	-
14.	Sucrose	-
15.	D-xylose	-
16.	Trehalose	-
17.	Sorbitol	-
18.	Malonate	+
19.	D-Arabinose	-
20.	Glycerol	+

Note: Positive (+); Negative (-)

Figure 1 illustrates the changes in pH recorded from first to fifth day of treatment with *Pseudomonas aeruginosa*. The pH of the medium was increasing from second day to fifth day of treatment. Increase in pH was noticed with the increase in treatment period and feather quantity like 0.8 and 1g.

An increase in pH was observed during feather degradation which is indicative of keratinolytic potential of microorganisms. Organism with higher keratinolytic activity turns media more alkaline in comparison with those exhibiting lower keratinolytic activities (18). This observation was based on the facts that keratin degradation involves oxidative deamination which results in production of ammonia and thereby increases the pH value. In this study increase in pH (above 8) was observed with the increase in treatment period and feather quantity. It has been observed that alkaline pH supports keratinase production and feather degradation in most microorganisms. The optimum pH for *B.altitudinis* GVC11 for keratinase production was found to be 9. Alkaline pH possibly favours keratin degradation because higher pH modifies cystine residues to lathionine, making it accessible for keratinase action [19].

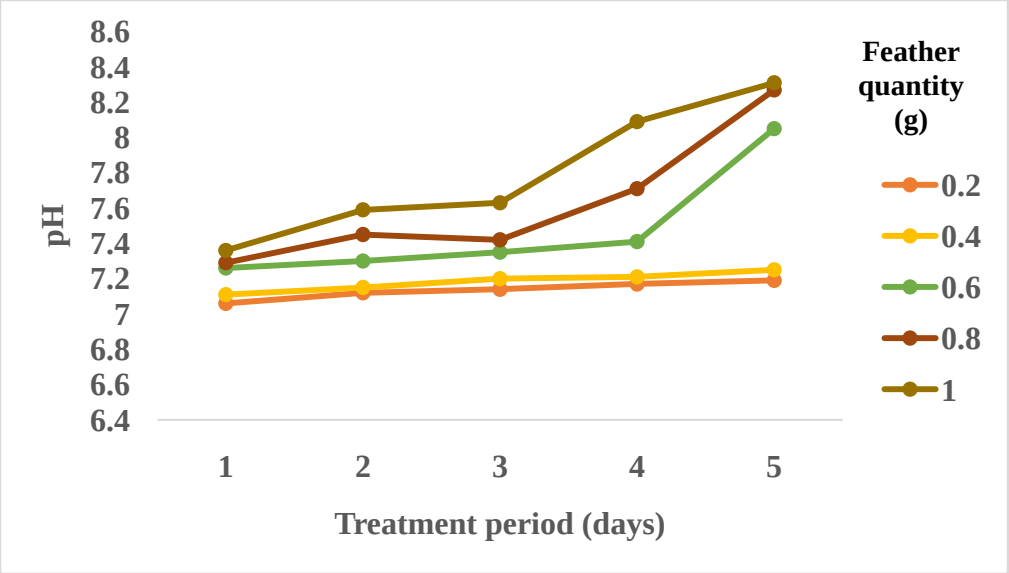

Fig.1 *Changes in pH during feather degradation by Pseudomonas aeruginosa*

Changes in the protein content of the medium during the five days of treatment of feather by *Pseudomonas aeruginosa* are depicted in Fig. 2. The biodegradation of feather resulted in the production of protein which is found to increase linearly with increasing quantity of feather. During degradation of one gram of feather more amount of protein was released on fifth day of incubation. Protein content exhibited an increase with the increase in treatment period and feather quantity. Maximum protein content was observed for one gram of feather after five days of treatment.

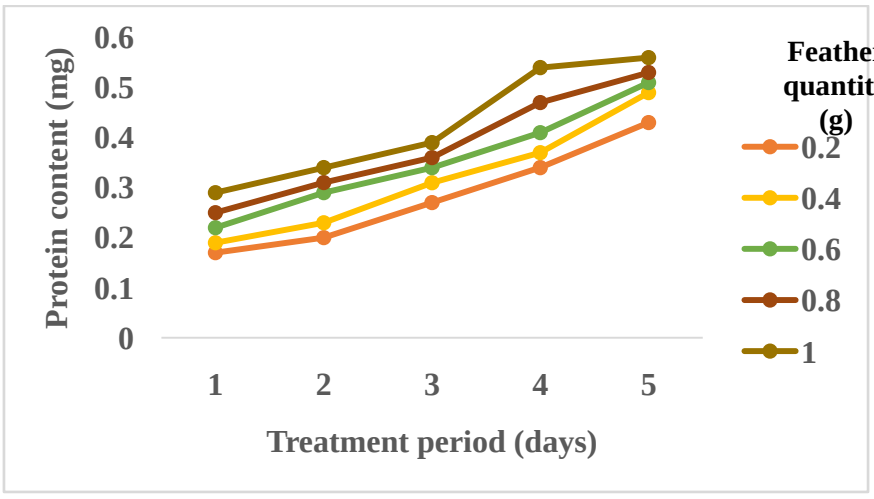

Fig.3 *Changes in protein content during feather degradation by Pseudomonas aeruginosa*

Figure 4 demonstrates the changes in the carbohydrate levels of medium during the five days of treatment of feather by *Pseudomonas aeruginosa.* The biodegradation of feather resulted in the trend of carbohydrate values fluctuating. During degradation of one gram of feather more amount of carbohydrate was released after two days of incubation. Maximum carbohydrate content was noticed for 0.2 gram of feather after four days of treatment while minimum was in one gram of feather after fifth day of treatment.

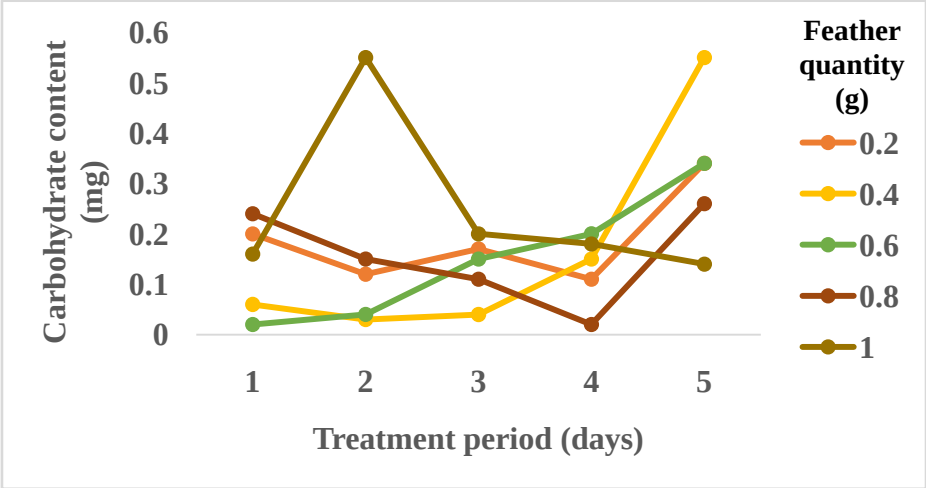

Fig.3 *Changes in carbohydrate content during feather degradation by Pseudomonas aeruginosa*

The protein content was high when compared to other animal wastes, with an average value of 81%. The ability to turn waste feathers into feed would reduces feed costs and it also reduces the amount of pollutants entering into the atmosphere and saves space in landfills which could be beneficial to the environment. Normally soy bean meal, corn, wheat middling and rice bran were used as feed. Compared to this feed, feather feed has more amount of protein and carbohydrate and also cheap [20].

The recent finding that B. licheniformis PWD-1 keratinase cause enzymatic breakdown of Prion protein PrP^{sc} [21] leave open a novel relevant application for broad range keratinase. Feathers constitute over 90% protein, the main component being beta-keratin, but it is tough to degrade. The poultry processing industry produces several million tons of feathers as a by-product, considering the high protein content; this waste could have immense potential as a source of protein for animal feed and for many other applications [22]. The protein content was high when compared to other animal waste, with an average value of 81% [23]. Feather hydrolysates produced by bacterial keratinase have been used as additives for animal feed [24]. Animal feed typically includes a carbohydrate source and a protein source.

Figure 5 exhibits the biomass of *Pseudomonas aeruginosa*during feather degradation. On fifth day, highest biomass was observed for one gram of feather. Feather quantity and treatment period dependent increase was observed in the biomass of *Pseudomonas aeruginosa.*

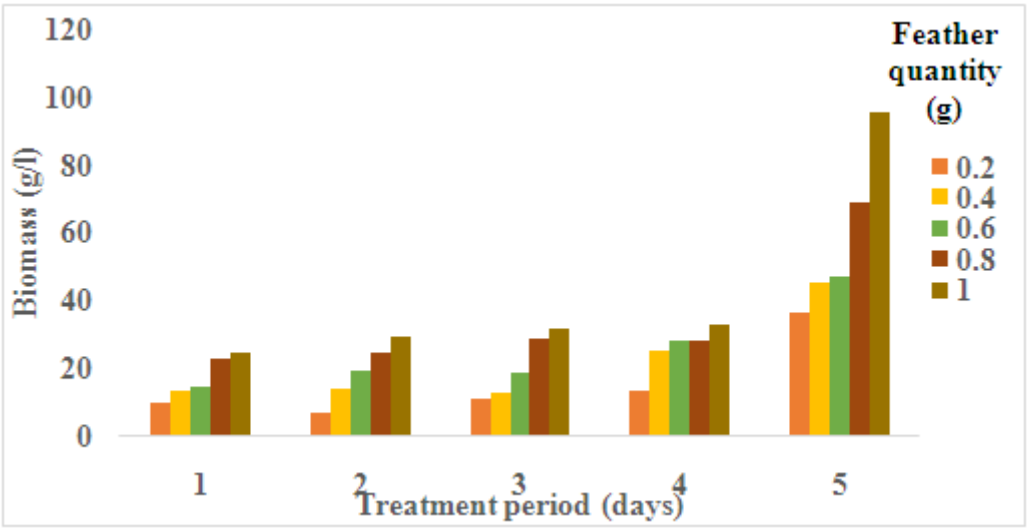

Fig.5 *Changes of Biomass during feather degradationby Pseudomonas aeruginosa*

The natural existence of keratin-degrading microorganisms offers more nutritionally balanced and digestible product. The study on the protein and aminoacid composition of the microbially treated and untreated feathers showed higher amount of lysine, methionine and arginine in the fermented feather, due to the possibility of secreting limiting amino acids by the microbes, which can also be a rich protein source [25].

Table 2 shows the two way ANOVA for pH, protein, carbohydrate and biomass with the variables treatment period and feather quantity. The variations due to feather quantity and treatment period were statistically significant at 5% level for all the four factors.

Table: 2. *Two way analysis of variance for the factors with the variables, treatment period and Feather quantity*

Factor	Source of variation	df	MS	Calculated F value	Table F value	Level of Significance at 5% level
pH	Feather Quantity	4	0.273074	7.587	3.006917	Significant
	Treatment period	4	0.401404	11.152	3.006917	Significant
Protein	Feather Quantity	4	0.064214	211.230	3.006917	Significant
	Treatment period	4	0.015334	50.440	3.006917	Significant
Carbohydrate	Feather Quantity	4	0.079514	0.769	3.006917	Significant
	Treatment period	4	0.084754	0.819	3.006917	Significant
Biomass	Feather Quantity	4	1510.74	24.348	3.006917	Significant
	Treatment period	4	570.09	9.188	3.006917	Significant

In the present study during the degradation of poultry feathers by*Pseudomonas aeruginosa*, pH, protein, carbohydrate content and biomass exhibited an increase with the increase in treatment period and quantity of feathers. This indicates that poultry feathers can be degraded by *Pseudomonas aeruginosa*for abating pollution by feathers and the mobilization of protein and carbohydrates towards animal feed preparation.

4. CONCLUSION

Pseudomonas aeruginosa was able to degrade poultry feathers confirmed by increase in pH, protein, carbohydrate content and biomass during the five days treatment.Itis a potential keratinolytic strain which is suitable for the bacterial degradation of keratin wastes and its fermentation broth could be useful in processes suitable for the conversion of feather to feed stock additives.

Conflict of Interests

The authors declare no conflicts of interest

ACKNOWLEDGMENTS

The authors thank the authorities of the American College for facilities and encouragement.

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