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Degradation of chicken feathers by *Leuconostoc* sp. and *Pseudomonas microphilus*

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ABSTRACT

Feather constitutes over 90% protein, the main component being beta-keratin, a fibrous and insoluble structural protein extensively cross linked by disulfide bonds. This renders them resistant to digestion by animal, insects and proteases leading to serious disposal problems. Keratinases which are produced by these keratinolytic organisms could be used to degrade feather waste and further the digested products could be an excellent material for producing animal feed, fertilizers or natural gas. Screened for the ability to hydrolyse kerterin in feather meal agar plates. Kekratinase activity of Pseudomonas microphilus maximum concentration 0.884 (20/ml) on 30 days and minimum concentration of 0.425 (IU/mt) on 30 days. The percentage of weight loss of feather treated for 10 days was found to be 20%. The percent weight loss of feather 20 days incubation was 45%. The weight loss of feather treated for 30 days was found to be 70% from the dates in Pseudomonas microphilus compare to Leuconostoc. sp.

INTRODUCTION

A total of 5-7 percent weight of mature chicken comprises of feathers. Feathers are composed of beta keratin [12] which is an insoluble protein and has a stable rigid structure because of several cross linking disulfide bonds involving cysteine. Keratin is also very rich in amino acids like Leucine and Serine. A number of feather degrading species of bacteria, actinomycetes and fungi have been used for the production of keratinase enzyme in submerged [2, 14] as well as in solid state fermentation. Most of keratinase enzymes from microbial sources are extracellular and inducible by keratin waste. There are several reports indicating the enzyme to be an alkaline protease [1]. The enzyme finds important application in leather industry [3].

Feather is a waste considerably generated from chicken processing industry. It becomes an animal supplement as feather meal by high temperature and pressure or chemical processes. Therefore, the meal has a low nutrition value since the process destroys certain amino acid. A biological approach could be advantages over the thermal and chemical methods since it is a friendly environment and energy conservation process resulting in its feather products could have a higher nutritional value served as a protein feed.

Bacterial strains are known which are capable of degrading feathers. These bacterial strains produce enzyme which selectively degrades the beta-keratin found in feathers. These energy make it possible for the bacteria to obtain

carbon sulfur and enzyme for that growth and maintaince from the degradation of beta keratin an enzyme capable of degrading protein is known as a protease and is described as having prateolytic activity. An enzyme which degrades keratin is a keratinize, while a beta-keratinizes is an enzyme capable of degrading beta-keratin. An enzyme which degrades keratin can also be described as having keratinolytic activity.

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 protein chains are packed tightly either in α-helix (α-keratins) or in β-sheet (β-keratins) structures, which fold into final 3-dimensional form [4, 7, 8]. Keratins are grouped into hard keratins (feather, hair, hoof and nail) and soft keratins (skin and callus) according to sulphur content [5,13]. This report describes the protein, aminoacid, keratinolytic activity and degradation of feather by *Pseudomonas microphilus* and *Leuconostoc* sp.

MATERIALS AND METHODS

Sample collection

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

Collection of culture

Leuconostoc sp. and *Pseudomonas microphilus* were collected from Microbial Germplasm Culture Collection Unit of Sri Gowri Biotech Research Academy, Tanjore.

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^{o}C for 24 hours in circulating hot airover. The weighed about 1g each and used for both test and control experiment.

Media preparation

Raw feather broth

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 such as *Pseudomonas microphilus* and *Leuconostoc.sp* was inoculated into respective medium. One 250ml of flask containing only the feather was maintained as control. These flasks were incubated at 37°C for 10, 20, and 30 days.

Recovers

After incubation period feather pieces were carefully removed from the culture (by using forceps) after different days of incubation. The collected pieces were washed throughly with tap water, ethanol and then distilled water. The pieces were shade dried and weighed for final weight. The data were recorded. The same procedure was also repeated for all the treated samples.

Determination of degradation of feather

The percentage of degradation of feather by *Pseudomonas microphilus* and *Leuconostoc.sp* were determined by calculating the percentage of weight loss of feather. The percentage of weight loss was calculating by the following formula.

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

Analytic method
Soluble protein concentrations were determined by the folin phenol reagent method [10] concentration of amino acids was determined by the Ninhydrin method [11].

Estimaion of keratinase activity
Keratinase activity was followed by the modified. The keratinolytic activity of the mixture containing 2ml Azokeratin (1% wW) and 0.5 ml suitably diluted enzyme was carried out at 45^{o}C for 30 min. The enzymatic reaction was stopped by adding with 2.5 ml of 10% TCA (Trichloro acetic acid) and then allowed to settle for 30 min, and then filtered. To 1 ml of the filterate 5ml of 0.5 ml sodium bicarbnonate solution and 0.5ml of diluted folin phenol cioculate reagent were added. After the reaction mixture was incubated for 30 min. The absorbance was measured at 660 nm using spectrophotometer. Simultaneously a blank read using the same steps expect that 10% TCA was added prior to the addition of enzyme. Results were expressed as keratinase units (kum-1g of enzyme).

RESULTS AND DISCUSSION

Screening for keratinolytic activity
Screening for feather degrading microorganisms was carried out from feathers in decomposition. Screened for the ability to hydrolyse kerterin in feather meal agar plates. Colonies producing clearing zones in this medium were selected for further work. Feather protein can be considered as an excellent source of metabolisable protein [9] and bacterial treated feathers may have similar nutritional feathers as soybeam meal production of digestible protein from low nutritional value by products may be an obtain one of the most expensive ingredients of diets.

Degradation of feather by *Leuconostoc sp*
The results of degradation feather by using *Leuconostoc*.sp. were presented in Table1, and fig 1. The percentage of weight loss treated for 10 days was found to be 10%. The percent weight loss of feather 20 days incubation was 24% similarity. The weight loss of feather treated for 30 days found to be 31 % from dates. It was very clear that feather degradation was maximum in sample subjected to long time incubation. After the first 10 days the sample incubated with *Leuconostoc* sp (Table-1&Fig-1).

Degradation of feather by *Pseudomonas microphilus*
The degradation of feather from bacteria was accessed on the percent weight loss of feather during treatment. After removed of the bacteria from feather strips range of microscopic changes was observed. In the caser of feather sillies incubated with *Pseudomonas microphilus* no weight loss was detected at the being of the experiments period (Table-1).

The percentage of weight lost increased significantly after two days of cultivation. The results of feather degradation of *Pseudomonas microphilus* were shown table-1. The percentage of weight loss of feather treated for 10 days was found to be 20%. The percent weight loss of feather 20 days incubation was 45%. The weight loss of feather treated for 30 days was found to be 70% from the dates. It was very clear that feather degradation was maximum in sample subjected to long time incubation.

Protein estimated by *Pseudomonas microphilus* and *Leuconostoc* sp.
The amount of protein estimated maximum concentrated of 0.548 mg/ml on 30 days present in *Pseudomonas microphilus* and 0.118 mg/ml on 30 days present in *Leuconostoc* sp (Table-2).

Estimation of Aminoacid by *Pseudomonas microphilus* and *Leuconostoc* sp.
The amount of amino acid estimated maximum concentration of 1.992 mg/ml on 30 days present in *Pseudomonas microphilus* and 1. 682, g/ml on 30 days present in *Leuconostoc* sp respectively (Table-3).

Keratinase estimated by *Pseudomonas microphilus* and *Leuconostoc* sp.
The activity of keratinase was followed during cultivation of strains in feather medium at 30°C. The biggest amount of keratinolytic activity produced was found in the supernamtant fluid of the culture medium.

Kekratinase activity of *Pseudomonas microphilus* maximum concentration 0.884 (20/ml) on 30 days and minimum concentration of 0.425 (IU/mt) on 30 days (Table-4). Previous investigated the increase in pH during cultivation is an important characteristic accompanying keratin hydrolysis and the keratinolytic potential of micro-organisms. The organisms with a higher keratinolytic activity turn the media more alkaline in comparison with other organisms exhibiting lower keratinolytic activity [6]. Selected bacteria strains have biotechnological potential for degradation and utilization of feather keratin.

Table-1: Feather degradation by using *Pseudomonas microphilus and Leuconostoc* sp.

S. No	Name of the organism	Days of treatment	Initial weight of feather (g)	Final weight of feather (g)	Percentage of weight loss (%)
1.	*Pseudomonas microphilus*	10 days	1	0.8	20
		20 days	1	0.55	45
		30 days	1	0.03	70
2.	*Leuconostoc* sp.	10 days	1	0.9	10
		20 days	1	0.76	24
		30 days	1	0.69	31

Fig-1: Feather degradation by using *Pseudomonas microphilus* and *Leuconostoc* sp.

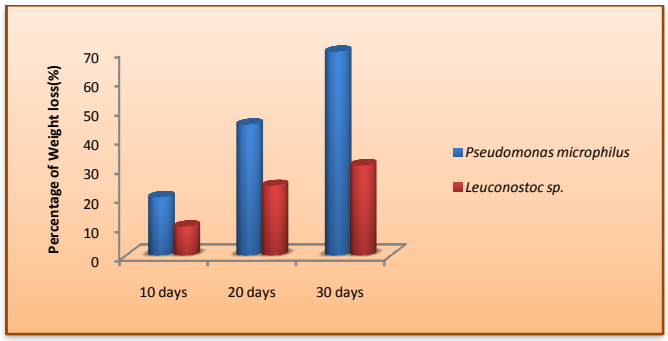

Table-2: Estimation of Protein

S. No	Name of the organism	Protein activity mg/ml		
		10 days	**20 days**	**30 days**
1.	*Pseudomonas microphilus*	0.275	0.398	0.548
2.	*Leuconostoc* sp.	0.068	0.105	0.118

Table-3: Estimation of Amino acid

S. No	Name of the organism	Amino acid activity mg/ml		
		10 days	**20 days**	**30 days**
1.	*Pseudomonas microphilus*	0.546	0.958	1.992
2.	*Leuconostoc* sp.	0.324	0.765	1.682

Table-4: Estimation of Karatinase

S. No	Name of the organism	Karatinase activity IU/ml		
		10 days	**20 days**	**30 days**
1.	*Pseudomonas microphilus*	0.458	0.674	0.884
2.	*Leuconostoc* sp.	0.197	0.310	0.425

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