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ORIGINAL RESEARCH ARTICLE

Degradation of Chicken Feathers by *Proteus vulgaris* And *Micrococcus luteus*

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ABSTRACT

Keratinase is an extracellular enzyme used for the bio degradation of keratin. Feathers comprise over 90% of proteins the main component being beta keratin, a fibrous insoluble protein containing disulfide bonds. These bonds provide resistant to digestion by the proteases of animals which are the major disposal problem for degradation of feather waste. Keratinase is the enzyme which is used to degrade feather waste and in the production of animal feed, fertilizers. The present study deals with comparison of feather degrading bacteria *Proteus vulgaris* and *Micrococcus luteus*. The present study screened for the ability to hydrolyse keratin in feather meal broth. Keratinase activity of *Proteus vulgaris* and *Micrococcus luteus* maximum concentration was 0.932(IU/ml) and 0.628(IU/ml) on 5 days respectively. The percent weight loss of feathers treated were found to be 26% ,68% and 79% at end of 2, 4 and 5 days respectively in *Proteus vulgaris* compared to *Micrococcus luteus.*

Key words: Keratinase, *Proteus vulgaris, Micrococcus luteus*, Lowery's method.

INTRODUCTION

Keratin is a highly stable insoluble protein present in hair, feather, nails, wool and horns [1]. A total 5-7% weight of mature chicken comprises of feathers which are composed of β-keratin. It is an insoluble protein of feathers and wool and is identified for their stability [2]. Keratin contains several cross linking disulfide bonds, responsible for the stability of keratin and its resistance to enzymatic degradation. Structurally, keratin polypeptide chains are packed tightly either in α-helix or in β-sheets which fold in 3-dimensional form [3]. Keratin protein distinctively has high sulphur content due to the presence of sulphur containing amino acids those are cystine, cysteine and methionine [4,5].Based on the sulphur content keratins are divided into hard keratin and soft keratin [6,7]. Feather is the waste obtained from chicken processing industry. Due to high protein content, keratin is used as good source of protein and amino acids by general recycling. Feather meal is used as animal feed obtained by subjecting to high temperature and pressure which destroys certain amino acids and hence has low nutritional value. Now the production of feather meal is by biological method, which is by using keratinase enzyme which produces higher nutritional protein feed [8].

Keratin is degraded by many species of bacteria, actinomycetes and fungi by producing keratinase enzyme. Keratinase is the enzyme produced by the microbes in the presence of keratin as substrate. The keratinase producing microorganisms have the ability to degrade chicken feather, hair, nails, wool etc. Keratinase enzyme was produced by different bacteria like *Bacillus subtilis* [9], *Bacillus licheniformis* [10], *Bacillus pumilis* [11], *Burkholderia, Arthrobacter* [12], *Pseudomonas, Microbacterium* [13] species. This enzyme is also isolated from fungi *Aspergillus niger, Streptomyces fradiae* [14], *microsporum sp, Onygena sp, Absidia sp*,some species of dermatophytes, including *Trichophyton sp, mentagrophytes, T. rubrum, T. gallinae, Microsporum canis and M. gypseum.*

Keratinase is a potential enzyme for removing hair and feather in the poultry industry for production of feather meal, clearing the obstructions in the sewage systems, de-hairing process in leather industry [15] and in the industries

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like cosmetics, diagnostics and pharmaceuticals [16,17,18]. The present study describes the comparison of proteins, amino acids and keratinolytic degradation of feathers by *Proteus vulgaris* and *Micrococcus luteus.*

MATERIALS AND METHODS

Sample collection

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

Culture collection

Micrococcus luteus and *Proteusvulgaris* cultures were obtained from National Collection of Industrial Microorganisms (NCIM), Pune.

Screening on skim milk agar plates

The *Micrococcus luteus* and *Proteus vulgaris* were checked for their proteolytic activity on skim milk agar plates. The organisms were streaked on milk agar plates incubated at 37ºC for 24hrs.

Surface sterilization of feathers

The raw feathers were cut into small pieces, cleaned with tap water to remove the dust particles, sterilised with 0.1% mercuric chloride and alcohol. Later rinsed with distilled water and dried at 45ºC for 24 hours in a hot air oven.1 gram of the feather sample was weighed for experimental tests.

Preparation of media

Raw feather broth was used for the fermentation of feather degrading microbes. The composition of the media:

Ingredients	Amount (gms)
Sodium chloride	0.5
Magnesium chloride	0.24
Ammonium chloride	5.5
Potassium dihydrogen phosphate	0.4
Dipotassium hydrogen orthophosphate	0.3
Yeast extract	0.1
pH	7.5

1000ml of raw feather broth was prepared and sterilized by autoclaving at 121^0 C for 15min. To 100ml of media, aseptically sterile pre-weighed feather pieces were added. Three flasks were inoculated with organisms *Proteus vulgaris* and *Micrococcus luteus* and incubated in an orbital shaker at 100rpm at 37^0C for 2, 4 and 5 days respectively.

Recovers

After different days of incubation period feather pieces were carefully removed from the culture (with forceps) media and washed thoroughly with water, ethanol and then with distilled water.The sample was dried,weighed and the data was recorded for all the samples at regular time intervals.

Determination of degradation of feather

The percentage of degradation was determined by calculating the percent weight loss of feather which was calculated by using the following formula.

$$\text{Percent weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}$$

Protein determination

Protein content in the broth was analyzed by using lowery method [19] with bovine serum albumin as standard protein.

Determination of amino acids

Amino acid content in the broth was analyzed by using the Ninhydrin method.

Estimation of keratinase activity

For 2ml of *azokeratin* (1%w/v), 0.5ml of diluted enzyme was added, incubated for 30min at 45^0 C. The enzymatic reaction was stopped by adding 2.5ml of 10% TCA (Trichloro Acetic Acid) and the allowed to settle for 30min and then filtered. To 1ml of the filtrate add 5ml of 0.5ml sodium bicarbonate solution and 0.5ml of diluted folin phenol cioculate reagent and incubated for 30min. The absorbance was measured at 660nm using spectrophotometer by using blank.

RESULTS

Screening on skim milk agar plates

The *Proteus vulgaris* and *Micrococcus luteus* strains produced clear zones in the medium. Their photos are recorded.

Degradation of feather by *P*roteus vulgaris and *M*icrococcus luteus

The feather degradation by *Proteus vulgaris* and *Micrococcus luteus* was graphically constructed in (**Fig 1**).The percentage of weight loss treated for 2,4,5 days was 26% ,68% and 79% respectively by *Proteus vulgaris* from date. The percentage of weight loss feather treated for 2,4,5 days 19%, 45% and 63% respectively by *Micrococcus luteus* .It was clear that feather degradation was maximum in sample subjected to long time incubation.

Estimation of protein

The amount of protein estimated in 5 days incubated broth with *Proteus vulgaris* gave maximum concentration of 0.682 mg/ml and 0.527 mg/ml for *Micrococcus luteus*. The results were constructed graphically in (**Fig 2**).

Estimation of amino acids

The maximum concentration of amino acids in the 5 days incubated broth of *Proteus vulgaris* was

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2.328mg/ml and for M*icrococcus luteus* was 1.621mg/ml. The results were constructed graphically in (**Fig 3**).

Estimation of Keratinase

Keratinase is an exo-enzyme produced in the medium was found in the supernatant. The keratinolytic activity of 2nd, 4th and 5th days of incubation was estimated by using keratin enzyme and azo-keratin. The keratinolytic activity of P*roteus vulgaris* was 0.932IU/ml and for the *Micrococcus luteus* was 0.628IU/ml. The organisms which are having higher keratinolytic activity turn the media more alkaline than the other organisms which exhibiting lower keratinolytic activity and utilization of feather keratin. The results were plotted in (**Fig 4**).

(**Fig 5**) showed day one inoculated cultures of *Micrococcus luteus* and *Proteus vulgaris* respectively. (**Fig 6**) showed 5th day cultures of *Micrococcus luteus* and *Proteus vulgaris* respectively

Fig 1: Graphical representation of feather degardation by *Proteus vulgaris* and *Micrococcus luteus*

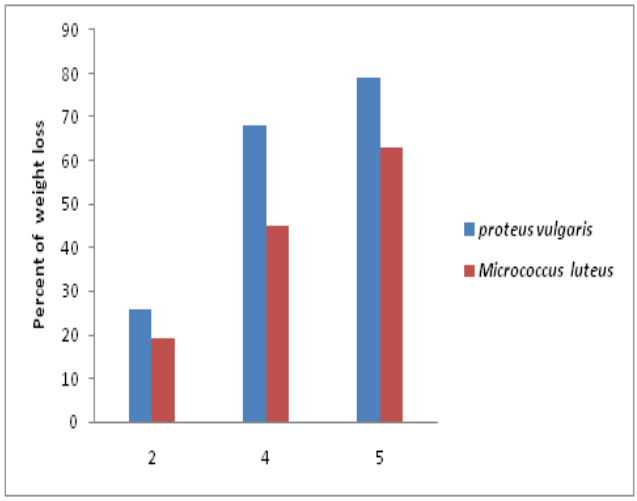


Fig 2: Estimation of proteins

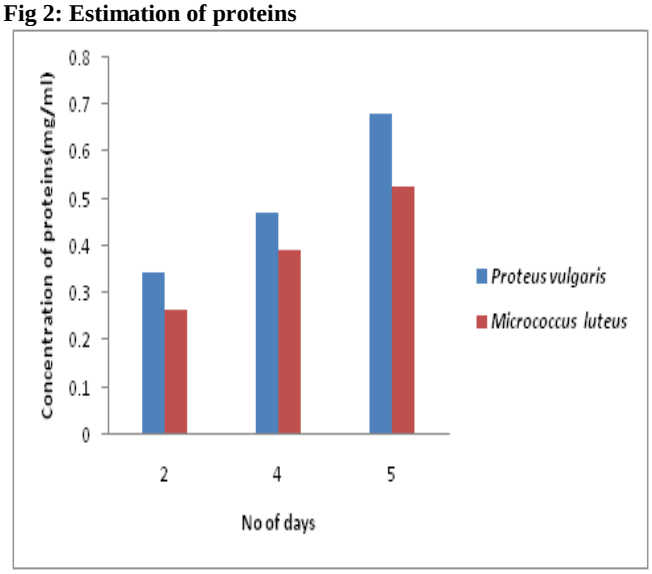


Fig 3: Estimation of aminoacids

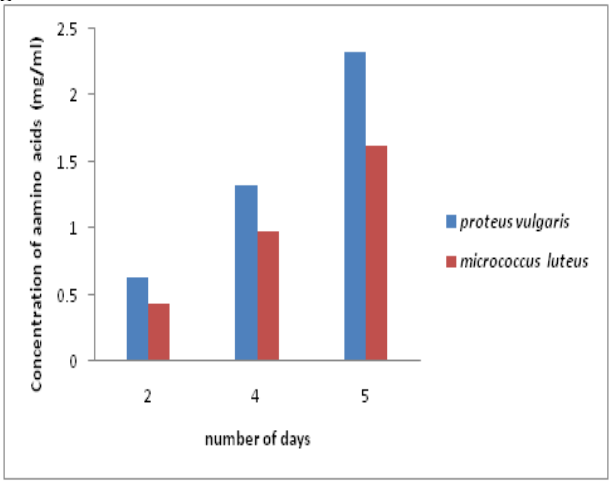
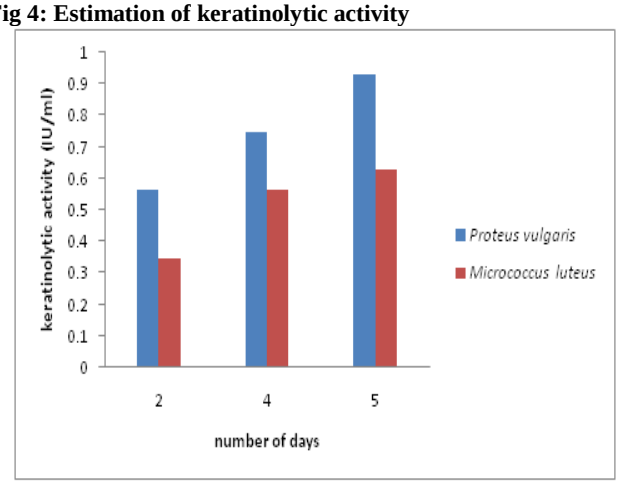


Fig 4: Estimation of keratinolytic activity



5 (A).Day one culture of *Micrococcus luteus*

5 (B).Day one culture of *Proteus vulgaris*

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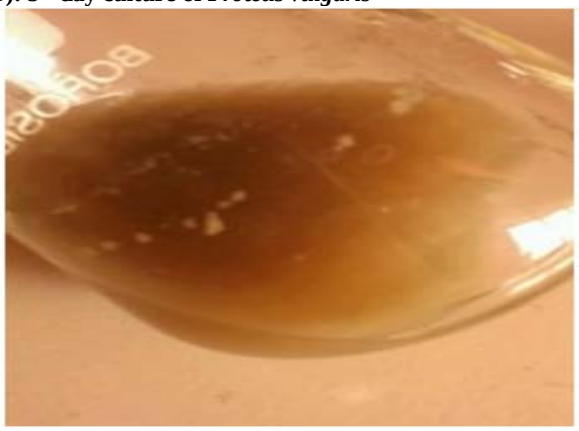
6 (A): 5th day culture of *Micrococcus luteus*

6 (B): 5th day culture of *Proteus vulgaris*



CONCLUSION

Finally it is concluded that *Proteus vulgaris* showed faster degradation of chick feathers than *Micrococcus luteus*. The percent weight loss of feathers treated was 26%, 68% and 79% at end of 2, 4 and 5 days respectively in *Proteus vulgaris* compared to *Micrococcus luteus*. The total amount of proteins, amino acids present and keratinolytic activity was estimated.

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