Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [5] 2022 :772-776

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Production of Keratinase from Feather Degrading Microorganisms from Poultry Soil

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ABSTRACT
Feathers are byproduct waste of poultry processing plant and produced in large amount. A small percentage of feather waste is steamed, chemically treated, ground, to form feather meal a dietary protein supplement for animals. Alternatively, keratin can be biodegraded by some Keratinolytic bacteria and in this study, Keratinase producing bacteria and their Keratinolytic enzyme production was investigated. Soil sample was collected from three different poultry form in trichy, mathur, TVS tollgate. Soil sample were inoculated in three enrichment media and colonies producing clear zone in feather meal agar were selected and identified as Bacillus licheniformis. It was able to degrade chicken and pigeon feathers. They produce extracellularly Keratinolytic enzymes in enrichment media with 10% Feather meal powder. We report that Keratinase and Protease activity were detected in the culture supernatant and optimal medium for extracellular production of Keratinase and Protease is feather meal media at pH (7) and temperature (37°C). The keratinous waste can be biologically degraded by enzymes or the microbe itself to form useful products.
Keywords: *Bacillus licheniformis, feather, Keratinolytic enzymes, feather meal,protease.*

Received 24.10.2022 Revised 26.11.2022 Accepted 18.12.2022

INTRODUCTION

Worldwide 24 billion chickens are killed annually and around 8.5 billion tonnes of poultry feather are produced. According to a recent report in leading news paper
India's contribution alone is 350 million tonnes. The poultry feathers are dumped, used for land filling, incinerated or buried, which involves problems in storage, handling, emissions control and ash disposal. Discarded feather also causes various human ailments
including chlorosis, mycoplasmosis and fowl cholera [1].Keratin is insoluble protein macromolecules with very high stability and low degradation rate. Keratin is mainly present in hair, nails, feather, wool and horns (A.A.Onifade *et al*., 1998). High protein content of keratin waste can be used as a good source of protein and amino acids by systemic recycling. Recycling of feather can provides a cheap and alternative protein feed stuff. Further this can be used for animals feed and many other purposes [2-4].Keratins (KRTs) are most abundant proteins in mammalian epithelial cells and most components of skin, nail, hair, horn, feather and wool. Two types of keratins, α-KRTs and β-KRTs, consist of tightly packed protein chains in α-helices and β-sheets, into super coiled polypeptide chain. Due to their extremely rigid structure KRTs are insoluble and hard to degrade. However certain microorganisms are able to degrade keratins by synthesis of keratinases [5].A new feather-degrading bacterium was isolated from a local feather waste site and identified as *Bacillus licheniformis* based on morphological, physiochemical, and phylogenetic characteristics. *Bacillus licheniformis* is a rod-shaped, Gram-positive bacterium. It tends to form spores in soil, which makes it desirable to be used for the industrial purposes such as the production of enzymes, antibodies, and small metabolite [6].The use of feather-lysate from *Bacillus licheniformis* with amino acids supplementation produced a similar growth rate in chickens when compared to chickens fed with a diet that including soyabean meal. Biodegradation of feathers by keratinase from microorganisms may provide a viable alternative. *Bacillus* [7-9] fungi [10,11] and actinomycetes [12,13] have previously been shown to be able to produce feather-degrading keratinases. In the current study we focused on the isolation and characterization of extracellular producing bacteria from the soil of feather processing units in Trichy, TN, India.

MATERIALS AND METHODS

Isolation of keratinolytic microorganisms: Soil was collected from a regular feather dumping site of Trichy, Mathur, TVS tollgate poultry processing plant, Tamil Nadu in sterilized sampling bags. Chicken feather were extensively washed in tap water and finally with double distilled water. The cleaned feather was cut into small pieces (0.2cm). Then boiled at 30-40 psi for 2-3h and allowed to cool Feathers were dried under sunlight and then in hot air oven at 60°C for 48h. They were stored at 5°C for further work.

Culture media:

The basic medium used for isolation and fermentation of the feather-degrading microorganisms contained the following constituents (g/l): NaCl (0.5), KH_2PO_4 (0.7), K_2HPO_4 (1.4), $MgSO_4$ (0.1), and feather (10), pH 7.2. Cultivation was done using 250 ml Erlenmeyer flasks containing 100ml medium and 20g/l of agar was used for screening the microorganisms in plates. For the medium used for screening mutants, 10 g casein was used instead of feathers. Luria-Bertani (LB) medium (peptone 1% (w/v), yeast extract 0.3% (w/v), NaCl 0.5% (w/v), pH (7.2) was used for inoculum preparation and isolate maintenance.

Screening medium:

The bacterial isolates were inoculated in the basal medium enriched with chicken feather waste. The pH was adjusted to 8.0. The medium was poured into petriplates in aseptic condition. After the media had been solidified, a loopful of culture was inoculated (simple streak) onto plate. Then the plate was incubated for 24 hrs at 37° C.

Identification and screening of keratinolytic bacteria:

Cultural characterization

The isolates were observed under the microscope, the colony morphology was noted with respect to color, shape, size, nature of colony and pigmentation [11].

Microscopic observation

The bacterial isolates were Gram stained and observed under a high power magnifying lens in light microscope. Endospore staining and motility test were perform to observe the morphology and motility of the cells [11].

Biochemical characterization

The bacterial isolates were characterized biochemically by indole test, methyl red test, voges proskauer test, Simmons citrate test, catalase test, oxidase test, urease test [11].

Effect of pH

Feather meal broth medium (containing 0.25% feather) was prepared (pH 5, 6, 7 and 8). The bacterial isolate was inoculated in to the feather meal broth medium. Inoculated mediums were incubated at 37° C on a rotary shaker at 100 rpm for 7 days. Absorbance of the medium was measured using spectrophotometer at 680nm against the feather meal broth as blank [12].

Effect of temperature

Feather meal broth medium was prepared and the bacterial cultures were inoculated into the Feather meal broth medium (containing 0.25% feather). Inoculated mediums were incubated at 27° C, 37° C, 47° C and 57° C on a rotary shaker at 100 rpm for 7 days. Absorbance of the medium was measured using spectrophotometer at 680nm against the feather meal broth as blank. After incubation over, both pH and temperature culture medium was filtered by filter paper. The filtrate was used for protein estimation by Lowry's method [12].

RESULT AND DISCUSSION

Keratin is a strong protein found in skin, hair, nails, horns, teeth. Keratin is difficult to dissolve due to the presence of cysteine disulfide that can form disulfide bridges. These disulfide bridges create an extremely strong helix shape. Microorganisms can degrade the keratin by the production of keratinase (an extracellular enzyme). Some bacteria, actinobacteria and fungi are reported to carry keratinolytic activity. In the current study bacteria was isolated from the soil samples collected from different feather processing areas in Trichy, TN, India.In the present study bacillus has been isolated, identified and classified by culture characterization, Microscopic observation and biochemical tests (Fig1).

It was found that the enriched feather degrading culture contained micro organisms exhibited keratinolytic activity. The feathers were fully solubilized within 10 days of incubation with the microbes from selected soil (Fig2).Keratinase enzyme activity was optimized with respect of pH and temperature. The results for pH effect are maintained in table 2 and fig3. The results for temperature effect are maintained in table 3and fig4. The bacillus isolate showed best enzyme activity at pH 7 and temperature 37°C.Result of this study indicates the potential keratinolytic organism can be used for the biodegradation of keratin in feather industries.

CONCLUSION

In this study a novel feather degrading from soil. The strain adapted to the soil environment such as temperature and enzyme activity. This study provided a scientific basis for the utilization of microbial resources in soil for the degradation of waste feathers.

Figure 1: Colony morphology of isolates

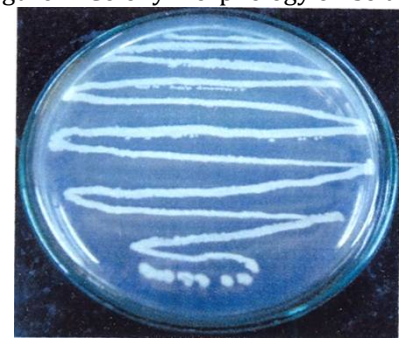


Figure 2: Showing organism degrading feathers efficiently

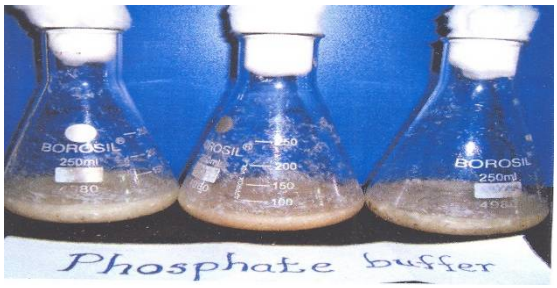


Table 1. Characterization of keratinolytic bacteria

Bacterial isolate	OD value at 680nm			
Bacillus sp	pH5	pH6	pH7	pH8
	0.54	0.56	0.69	0.51

Culture character Colony morphology large, round, irregular, fast growing colonies

Microscopic characters Gram staining Gram positive rods

Motility Motile

Biochemical characters

Indole	Negative
Methyl Re	Positive
Voges Proskaue	Negative
Citrate utilization	Positive
Catalase	Positive
Oxidase	Positive
Urease	Negative

Table2: Effect of different pH on enzyme activity.

Bacterial isolate	OD value at 680nm			
Bacillus sp	27°C	37°C	47°C	57°C
	0.68	0.79	0.53	0.31

Fig3: Effect of different pH on enzyme activity

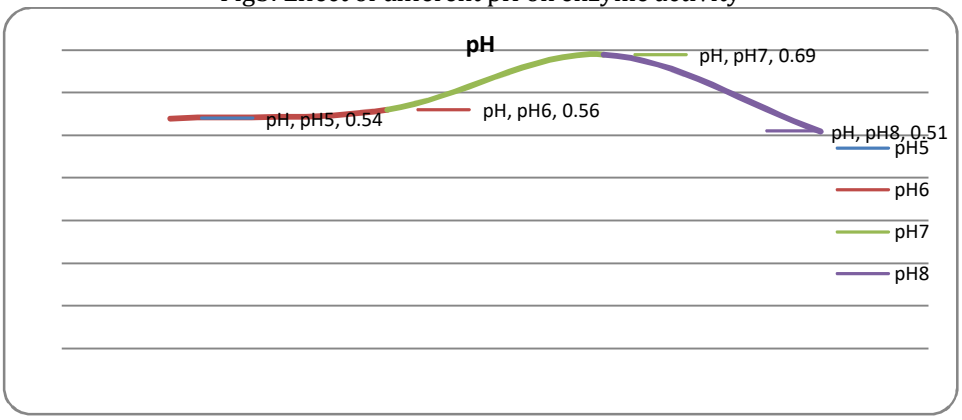
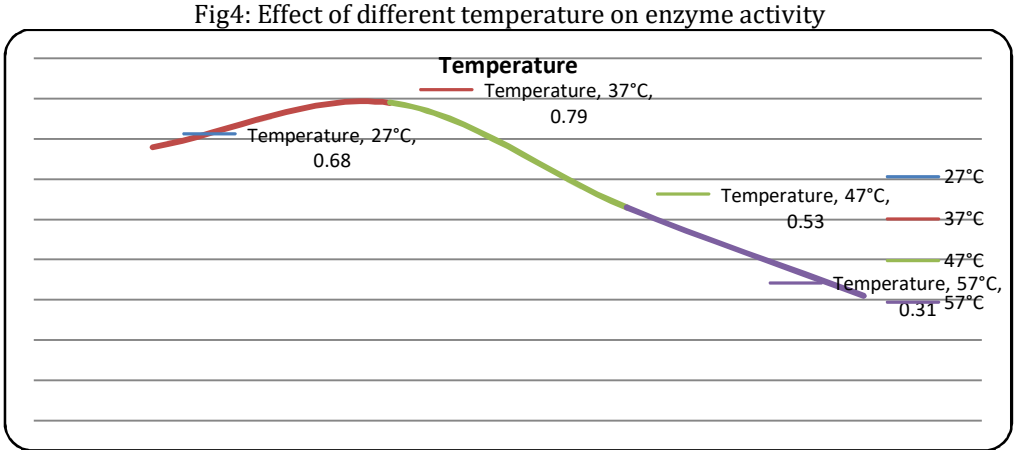


Fig4: Effect of different temperature on enzyme activity



ACKNOWLEDGEMENT

The authors acknowledge Chairman A. Srinivasan, Dhanalakshmi srinivasan college of arts and science for women (A) for financial support of this work.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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CITATION OF THIS ARTICLE

R. Madura, T. Sheela, Dhanabalan.R Nandhini.M and Jaya Singh: Production Of Keratinase From Feather Degrading Microorganisms From Poultry Soil. Bull. Env.Pharmacol. Life Sci., Spl Issue [5]: 2022: 772-776.