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Research Article

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Keratinloytic Actinomycetes Isolated From Poultry Waste

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ABSTRACT

Aim of this study was to isolate keratinolytic actinomycetes from poultry waste. Four soil samples were collected from three different poultry farm of Gwalior, Guna and Bhind district of MP, India. Soil suspension were prepared by serial dilution method, inoculated into two different media actinomycetes isolation agar and starch casein agar and incubated at 28°C for 7-14 days. Thirty five isolate were purified and obtained after screening on basis of their different morphology and colour of aerial and substrate mycelium. After gram staining all isolate were found gram positive. Biochemical characterization of gram positive isolate was also performed. Out of Thirty five isolates, twenty nine showed keratinolytic activity in primary screening on Skim milk agar plate, in which clear zone around the colonies were selected responsible for degradation of chicken feathers. Secondary screenings of twenty nine isolates were performed in modified liquid basal medium supplemented with feather for keratinolytic activity. Twenty seven isolates out of twenty nine, showed keratinolytic activity and able to efficiently utilized feather as source of carbon and nitrogen. All isolates were preserved on ISP1 broth (Tryptone – yeast extract Broth) and glycerol stock for storage. The study showed that isolates from poultry soil from 3 districts were able to degrade the keratin waste (feather) by their keratinolytic activity of keratinase enzyme.

Keywords: Keratinolytic activity, actinomycetes, poultry waste, keratinase.

INTRODUCTION

Feathers are produced in large amounts as a waste by-product of poultry processing plant. The poultry feathers are dumped, used for land filling, incinerated or buried, which involves problems in storage, handling, emissions control and ash disposal **[1].** Accumulation of feathers will lead to environmental pollution and feather protein wastage **[2, 3]**.

Keratins are the most abundant proteins in epithelial cells of vertebrates and present the major constituents of skin and its appendages such as nail, hair, feather, and wool **[4]**. Keratin wastes have high protein content, they could be used as a source of protein and amino acids for animal feed and any other applications. Keratin in its native state is highly stable structure due to tightly packed in helix and sheets into supercoiled polypeptide chain **[2, 5, 6, 7]**, that is not easily degraded even by other common proteolytic enzymes like trypsin, papain and pepsin.The composition and molecular configuration of keratin; its constituent amino acids, disulphide bonds, cross-linkages are responsible forhard to degrade and insolubility **[8, 5]**. Keratins are grouped into hard keratins (feather, hair, hoof and nail) and soft keratins (skin and callus) according to sulphur conten**t [9].**

A current value added use of poultry feathers are the conversion of feather to dietary meal for animal feed, by using physical and chemical treatments. These methods can destroy certain amino acids, decrease protein quality and digestibility of meal **[2, 10]**. Keratinolytic microorganisms and their enzymes may be used to enhance the digestibility of feather keratin meal. They may have important role in processing keratin-containing wastes from

poultry and leather industries through the development of non-polluting methods **[3]**. Biodegradation of feathers by keratinase from microorganisms may provide viable alternative sources. Various species of *Bacillus sp*. **[11, 12, 13, 14]**, fungi **[15, 16, 17]** and *Actinomycetes sp*. **[18, 11, 19]** have been reported for feather degrading activity by keratinase.

Ability of keratinolytic microorganisms to degrade keratin into economically useful keratin product **[20, 13, 21],** i.e. nitrogenous fertilizers, biodegradable films, glues and foils **[22, 23, 24]** are well known. It had been reported natural resources like plants and microorganisms involved in bioremediation and antimicrobial activity against pathogenic microorganisms **[25, 26].** Due to the involvement of microorganism in degradation of feathers encourage the biotechnologist and microbiologist to utilize their enzymatic ability in large scale for decomposing feathers, hairs, animal house wastes. Microbes play important part in carbon cycle in environment, by degradation of calcitrant compounds, keratin waste, non-degradable compounds. Study reveal the actinomycetes involved in degradation of keratin waste through its metabolism, is much safe than other commercial method currently in use and environment friendly also. Aim of the study is to identify and isolate the keratinolytic actinomycetes from poultry farms soil of Gwalior region.

EXPERIMENTAL SECTION

Collection of soil sample:
Soil samples were collected from four different poultry farms of Gwalior, Bhind and Guna district of Madhya Pradesh respectively. Soil samples were taken from the surface of poultry farms, where feathers are dumped. Samples were carrying to the laboratory in air tight sterile plastic bags.

Isolation of Actinomycetes:
Isolation of kerantinolytic actinomycetes from soil of poultry samples by serial dilution method. 1gm of soil was suspended in 9ml of distilled water and diluted upto 10^{-8}, prepared suspension was plated over AIA (with addition of glycerol 5 ml/l) **[27]** and SCA (pH-7.3) **[28]** supplemented with cycloheximide at the concentration of 50µg/ml as antifungal. Isolates were screened and purified by re-streaking. Plates incubated at 28°C for 7–10 days. Pure isolates were maintained ISP1 and stored at 4˚C for short term storage and in 20% glycerol stock for long term storage further characterization **[29]**.

Characterization:
Microscopic characterization of isolates was done by Gram Staining. Biochemical characterization of isolates done by following test; Amylase production test, Gelatinase production test, Cellulase production test (CMC), Urease production test, Catalase test, IMVIC test and Triple Sugar Iron test carried out **[30, 31].** Purified actinomycetes isolates were used for further experiment.

Screening of keratinolytic actinomycetes:
Primary screening of keratinolytic actinomycetes: Primary screening of isolates was performed on Skim Milk agar plate. The isolates were inoculated on skim agar plate and the plates were incubated at for 72 hrs. Clear zones around the isolate give positive result for keratinolytic activity **[13].**

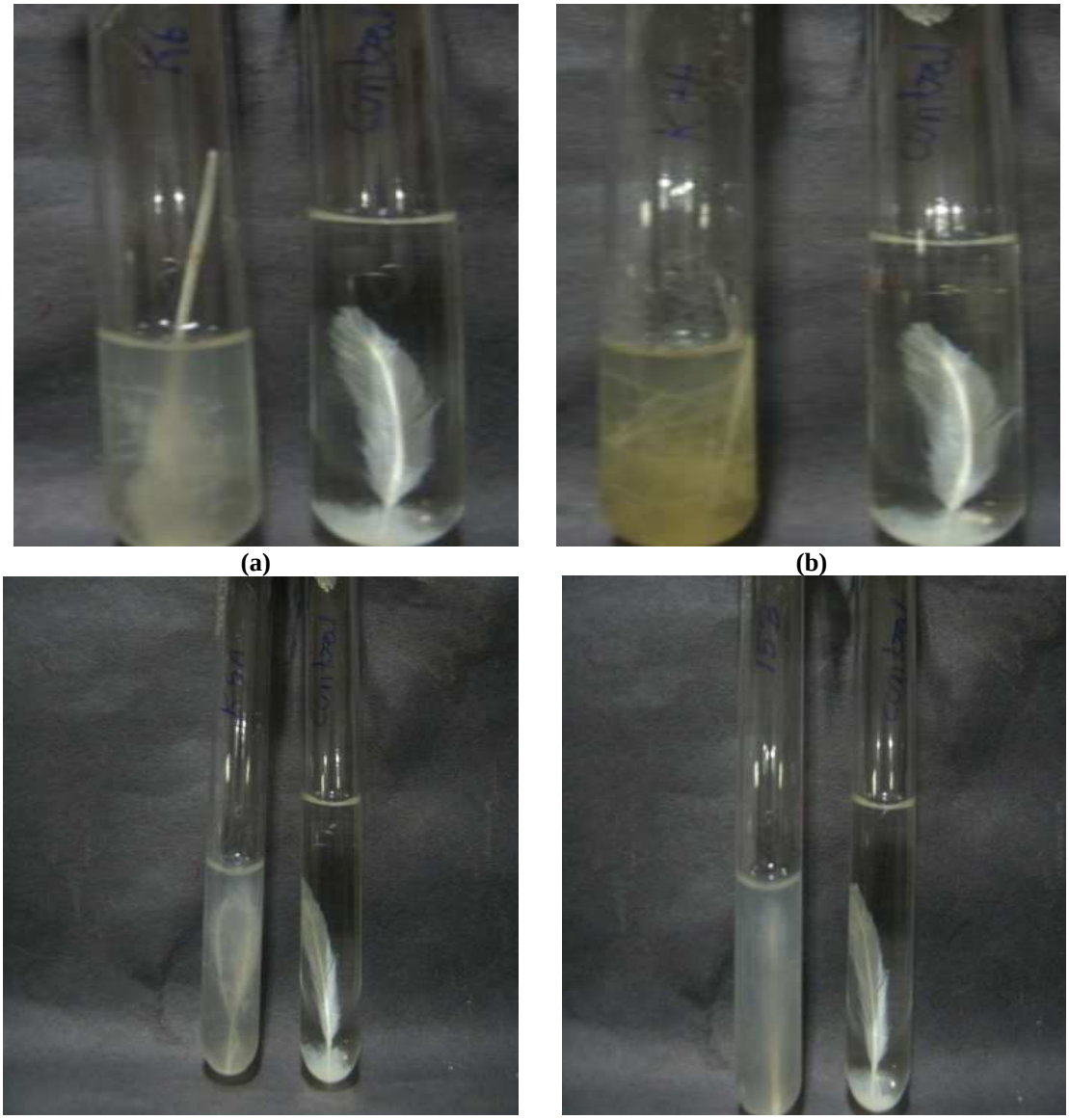
Secondary screening of keratinolytic actinomycetes:
All positive isolates screened from the primary screening, were subjected to secondary screening into the Modified basal liquid medium (K_2HPO_4 -1.5g, $MgSO_4.7H_2O$ - 0.05g, $CaCl_2$ - 0.05g, $FeSO_4.7H_2O$ - 0.015g, $ZnSO_4.7H_2O$ - 0.005 g/l) **[32].** The keratinolytic isolates inoculated into the modified basal liquid medium supplemented with raw chicken feather and incubate at 28±2°C. Raw chicken feather was collected from poultry farm, feathers were washed in running tap water and then completely dried at 60-65˚C in hot air oven.

RESULTS AND DISCUSSION

Total 35 isolate screened and purified from 4 poultry soil samples from 3 different districts Gwalior, Bhind and Guna of Madhya Pradesh, India. In gram staining all isolates were found gram positive. In Primary screening, totally 6 (Isolate AGW2, AKG1, AKG2, AKG3, AKG7 and AKG8) out of 35, did not showed keratinolytic activity on Skim Milk Agar Plate.

(a) **(b)**

Figure1. Screening of keratinolytic activity of poultry soil isolates on skim milk agar.



(a) **(b)**

(c) **(d)**

Figure2. Degradation of feathers in modified liquid basal medium inoculating with actinomycetes isolates.

Isolates were found positive in primary screening, selected for secondary screening. In Secondary screening 29 isolates were inoculated into modified liquid basal medium. Feather degradation or keratinolytic activities of isolates were visualized by naked eyes after 7 days of incubation in Modified liquid basal medium. Out 29 isolates; 2 isolate (ABD12, AGU16) did not showed feather degradation or keratinolytic activity and 3 isolate ABD14, ABD15 and

AKG12 showed moderate keratinolytic activity. Rest 24 isolates of poultry soil, showed high keratinolytic activity or feather degradation in modified liquid basal medium.

Table1. Keratinolytic activity of isolates on Skim milk agar and Modified Basal Liquid Medium

Isolates	Keratinolytic Activity	
	On Skim Milk Agar Plate	In Modified Basal Liquid Medium
AGW1	Positive	Positive
AGW2	Negative	Negative
AGW3	Positive	Positive
AGW4	Positive	Positive
AGW5	Positive	Positive
AGW6	Positive	Positive
AGW7	Positive	Positive
AGW8	Positive	Positive
AGW9	Positive	Positive
AGW10	Positive	Positive
ABD11	Positive	Positive
ABD12	Positive	Negative
ABD13	Positive	Positive
ABD14	Positive	Moderate
ABD15	Positive	Moderate
AGU16	Positive	Negative
AGU17	Positive	Positive
AKG1	Negative	Negative
AKG2	Negative	Negative
AKG3	Negative	Negative
AKG4	Positive	Positive
AKG5	Positive	Positive
AKG6	Positive	Positive
AKG7	Negative	Negative
AKG8	Negative	Negative
AKG9	Positive	Positive
AKG10	Positive	Positive
AKG11	Positive	Positive
AKG12	Positive	Moderate
AKG13	Positive	Positive
AKG14	Positive	Positive
AKG15	Positive	Positive
AKG16	Positive	Positive
AKG17	Positive	Positive
AKG18	Positive	Positive

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