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FIRST REPORT ON SCREENING OF KERATINOLYTIC BACTERIA FROM SOIL SAMPLES OF POULTRY WASTE DUMP SITES OF WIDE DELHI-NCR REGION

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ABSTRACT

Keratin is an insoluble and fibrous structural protein that is a constituent of feathers and wool. The protein is abundantly available as a by-product. The degradative product represents a valuable source of proteins and amino acids that could be useful for animal feeds or as a source of nitrogen for plants. However, the keratin-containing substrates and materials have high mechanical stability and hence are difficult to be degraded by common proteases. The present study involves the isolation of feather-degrading bacterial cultures isolated from soil samples of poultry waste dump sites of New Delhi. About 10 samples of soil were collected from various places of Delhi (Old Delhi railway station; Rohini West; Chandni Chowk; Ghazipur Mandi), Ghaziabad (Old Ghaziabad; Pratap Vihar) and Noida (Sector -4; 15; 53; 137) of Delhi-NCR region. The isolation of bacterial cultures was performed on Nutrient agar. The pure cultures were separately isolated in sterilized conditions and were maintained as glycerol stock for further use. The results showed the isolation of 25 pure bacterial cultures from soil samples. These bacterial cultures were able to grown in media with keratin/feathers as the carbon and nitrogen source. Further these bacterial strains were screened for keratinase production on keratin-agar plates. Amongst, 25 bacterial cultures, 12 bacterial cultures were found positive for keratinase production. These bacterial cultures were interpreted as keratinophilic bacterial straisn. Thus these 12 bacterial cultures were considered for the study and the reference codes were allotted to such bacterial cultures, NCFT/KB/101 to NCFT/KB/112. These bacterial cultures were further morphologically and biochemically identified and were screened for gram staining. The present study is the first kind of study ever reported on the isolation and screening of bacterial cultures for keratinase production from soil samples of poultry waste dump sites of wide Delhi-NCR region.

KEYWORDS: Bacterial strains, poultry waste dump sites, keratinase production, keratinolytic, Delhi-NCR region.

INTRODUCTION

Feather wastes are generated in large quantities as a byproduct of commercial poultry processing which represent 5-7% of the total weight of mature chickens. These are made up primarily of keratin, which is also found in the claws and armour of reptiles and the hooves, horns, hide, hair and nails of mammals. These feathers constitute a sizable waste disposal problem. Several different approaches have been used for disposing of feather waste, including land filling, burning, natural gas production and treatment for animal feed. Most feather waste is land filled or burned, which involves expense and can cause contamination of air, soil and water. Keratin, by virtue of its insolubility and resistance to proteolytic enzymes, is not attacked by most living organisms. Nevertheless, keratin does not accumulate in nature and, therefore, biological agencies may be presumed to accomplish its removal. Several insects, including clothes moth larvae, carpet beetles and chewing lice are known to digest keratin. The common occurrence in nature of microorganisms that readily and, in some cases, preferably grow on keratinaceous substrates has supported the general belief that certain microorganisms can digest keratin.[1] Insoluble and hard-to-degrade animal proteins are ubiquitously present throughout animal bodies. Enormous numbers of these proteins are generated in the meat industry in a mixture of bones, organs and hard tissues, finally being converted to industrial wastes, the disposal of which is tremendously difficult. Most animal proteins (feathers) are currently disposed of by incineration. This method, however, has ecological disadvantages in terms of an apparent energy loss and the production of a large amount of carbon dioxide. Thus, an innovative solution to these problems is urgently needed.[2] Bacterial strains are known which are capable of degrading feathers. These bacterial strains produce enzymes which selectively degrade the beta-keratin found in feathers. These enzymes make it possible for the bacteria to obtain carbon, sulfur and energy for their growth and

maintenance from the degradation of beta keratin. An enzyme capable of degrading protein is known as a protease and is described as having proteolytic activity. An enzyme which degrades keratin is a keratinase, while a beta-keratinase is an enzyme capable of degrading beta-keratin. An enzyme which degrades keratin can also be described as having keratinolytic activity. Keratinases from bacteria are isolated and characterized. For instance, keratinase from *Bacillus sp*.[3] *Bacillus licheniformis*[4-7], *Burkholderia*, *Chryseobacterium*, *Pseudomonas*, *Microbacterium sp*[8], *Chryseobacterium sp*.[9,10], *Streptomyces sp*.[11,12] were isolated and was studied with respect to various parameters. Keratinases are a group of serine metalloproteases, release the free amino acids from keratinous proteins. Keratin is resistant to the common proteolytic enzymes, papain, pepsin and trypsin.[13] These enzymes have been studied for dehairing processes in the leather industry [14] and hydrolysis of feather keratin[15], which is a by-product generated in huge amounts by the poultry industry. Discarded feathers are currently used to produce feather meal through thermal processing, resulting in a low nutritional value product.[16]

MATERIALS AND METHODS

The chemicals and reagents used in the present study were of analytical grade procured from Ranchem, Mumbai, India and media was procured from Hi-Media, Mumbai.

Collection of soil samples from poultry waste dump sites

Survey of different sites of the poultry waste dump sites of wide Delhi-NCR region was done for collection of soil samples. The soil samples were collected from poultry waste dump sites of Delhi (Old Delhi railway station; Rohini West; Chandni Chowk; Ghazipur Mandi), Ghaziabad (Old Ghaziabad; Pratap Vihar) and Noida (Sector -4; 15; 53; 137) of Delhi-NCR region. Nearly about 500 g of soils was collected from each site by using sterilized spatula and kept in sterilized sealed polythene bags duly labeled by permanent ink marker. These samples were brought to the laboratory, kept in refrigerator till the final analysis.

Sterilization of glasswares and miscellaneous items

Cleaning and sterilization of glasswares and minor equipments was done in each case. Borosil and Corning's glasswares were used for all the laboratory experimental studies. The glasswares used for the experiment was thoroughly washed with liquid detergent (Cedepol) and then sun dried. After sun drying these glasswares was again cleaned by keeping them in chromic acid over night after chromic acid treatment they was washed with distilled water to remove chromic acid and again sun dried. After drying, these glasswares was sterilized by keeping them in the oven at 160-180^{0}C for 4-6 hours. The small instruments like forceps, needles etc. was ordinarily sterilized by dipping them in 95% alcohol followed by flaming. These instruments were repeatedly sterilized during the operation to avoid contamination. The mouth of the culture vessels was also flamed before pouring or inoculation. Culture vessels containing the medium was plugged with cotton and sealed with aluminum foil and was generally sterilized by heating in an autoclave at 15 psi (Pound per square inch) at 121°C for about 20minutes. Before pouring the culture media or inoculation, the hands were repeatedly sterilized with 75% alcohol to avoid contamination.

Isolation and sub culturing of keratinophillic/keratinolytic bacteria

Soil samples were collected from a local poultry industry. Each of the soil samples (1 g) was flooded in saline solution 0.85%, suspension up to 10^{-5} was made and used to streak feather meal agar plates (10 g/l feather meal; 0.5 g/l NaCl; 0.3 g/l K2HPO4; 0.4 g/l KH2PO4; 0.1 g/l MgCl2- 6H2O; 0.1 g/l yeast extract; 15g/l agar, pH 7.5) which was incubated at 37^{o}C for 24 hours. [17] The organism screened with keratin agar plates was sub - cultured by continuously growing the bacterium in basal broth medium (4 days at 37°C, 120rpm) and subsequently streaking on basal agar medium (2% agar, 2 days 37°C).

Morphological characterization and biochemical tests for identification of isolated bacteria

Bacterial identification was conducted on morphological, physiological and different biochemical tests. Results were further compared with Bergey's Manual of Determinative Bacteriology, 9th edition.[18]

Screening of isolated strains for keratinase production by plate assay

The isolates were screened for keratinase activity. This was done by inoculating the broth culture of organisms on the feather powder agar plates containing 0.4% feather powder [washed feathers was dried at 50^{o}C in a forced draught. The dried feathers were ground into fine fractions, incubated at 37^{o}C for 48 hours. A clear zone around the growth of the bacteria was indicated and determined as positive keratinase producers.[19]

RESULTS

In the present investigation, total of 25 bacterial cultures were isolated from soil samples of poultry waste dump sites of Delhi-NCR region **(Figure 1).** The screening of these bacterial cultures for keratinase production confirmed that 12 bacterial strains are the significant producers of keratinase enzyme. The results are shown in **Figure 2**. These bacterial cultures were thus considered for the study and were further identified by different biochemical tests and gram staining. The strains were differentiated on the basis of their biochemical characteristics; however specific genus representation of the isolates was performed and recorded. The results are shown in **Table 1**.

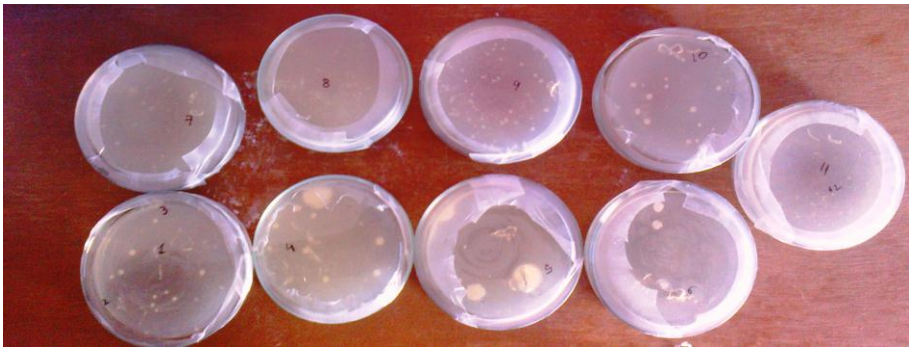

Figure 1: Isolation of bacterial cultures on nutrient agar medium from soil samples of poultry waste dump sites.

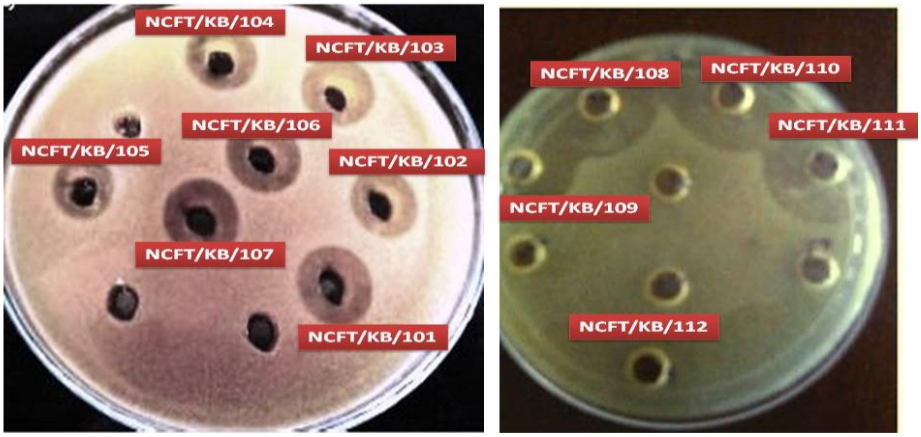

Figure 2: Keratinase production by isolated keratinophilic bacterial strains.

Table 1: Biochemical and gram staining tests performed for keratinophilic bacterial strains.

Isolate (NCFT/KB -xxx)	Gram Staining /Shape/Motility/Spore formation	Sugar Fermentation Test	Amylase test	Indole test	Methyl red test	VP test	H_2S production	Citrate test
101	Gram positive, Bacilli, Non-motile, spore forming (Bacillus sp.)	+	+	+	+	-	_	-
102	Gram positive, Bacilli, Non motile, Non spore forming (Bacillus sp.)	+	+	_	+	-	_	-
103	Gram positive, Bacilli, Non motile, Non spore forming (Bacillus sp.)	+	+	_	+	+	_	-
104	Gram positive, Bacilli, Non motile, Non spore forming (Lactobacillus sp.)	+	+	_	+	-	-	-
105	Gram positive, Bacilli, Non motile, Non spore forming (Lactobacillus sp.)	+	+	_	+	+	-	-
106	Gram negative, Bacilli, motile, Non spore forming (Lactobacillus sp.)	+	+	+	_	+	+	+
107	Gram negative, Bacilli, motile, Non spore forming (Lactobacillus sp.)	+	+	+	_	+	_	+
108	Gram negative, Bacilli, motile, Non spore forming (Lactobacillus sp.)	+	+	+	+	+	+	+
109	Gram positive, cocci in chains, non motile, spore forming (Streptococcus sp.)	+	+	_	+	-	-	-
110	Gram positive, cocci in chains, motile, spore forming (Streptococcus sp.)	+	+	+	+	-	-	-
111	Gram positive, cocco-bacilli, motile, non spore forming (Acinetobacter)	+	+	_	+	-	-	-
112	Gram positive, cocci, non motile, spore forming (Staphylococcus sp.)	+	+	_	+	-	-	-

*+, showed; -, don't showed.

DISCUSSION AND CONCLUSION

The present study is a short communication determining the isolated keratinophilic bacterial cultures from the soil samples collected from poultry waste dump sites of Delhi-NCR region. The isolation of different bacterial cultures from the samples showed that there is an enriched and unexplored biodiversity of microbes in such areas where nature is responsible for keratin degradation. The isolated bacterial strains perhaps will be useful to enrich the previous data base on diversity of bacterial strains isolated from poultry waste dump sites. Different researches have reported the existence of different bacterial colonies in keratin diversified regions.[20, 21]

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