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Screening and Isolation of Keratinase Producing Bacteria from Poultry Waste

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Abstract

This work has been undertaken for the Screening and isolation of Keratinase producing strains of Bacteria were carried out from Ten soil samples, collected from various regions of Bangalore and used to screen for Keratinase production by using feather powder agar plate assay. In the present study, an attempt was made to isolate efficient Keratinase producing bacteria from diverse poultry waste. Different isolates were screened for possessing the ability to produce Keratinase. About 6 bacterial isolates were found to be promising to produce Keratinase. The organisms were tested for various biochemical tests, which leads to their identification as *Bacillus licheniformis, Bacillus cerus,* and *Staphylococcus aureus.*

Keywords: *Keratinase, feather powder, poultry waste, Bacillus licheniformis, Bacillus cerus , Staphylococcus aureus.*

Introduction

Enzymes are delicate protein molecules necessary for life. Proteases are the single class of enzymes which play an important part in the metabolism of almost all organisms (Plants, Animals, Fingi, Bacteria and Viruses).Investigation of proteases is a central issue in enzymology due to their wide applications in Landry detergents, Pharmaceutical. Leather products, Photography, Food, Agricultural products and Bioremediation process. Among the various proteases, bacterial extracellular proteases are the most significant, compared with animal,Plants,viruses and fungal extracellular proteases. Extracellular proteases produced by Bacillus and cocci species are of main interest from a biotechnological perspective, and are not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as detergents, foods, tannery, pharmaceutical and leather industries. These proteases account for 60% of the total worldwide production of enzymes. The genus Bacillus and cocci contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the srtains of Bacillus and cocci. Keratinase (EC 3.4.4.25) belongs to the class hydrolase which are able to hydrolyse insoluble keratins more efficient than other proteases and their action are very specific, i.e., they acts only on keratin substrates, Keratin are insoluble fibrous proteins found in hair, wool, feather, nail, horns and other epithelial covering which is rich in beta helical coil linked through cysteine bridges. Keratinases are hydrolyze both native and denatured keratin, and the enzymes are widely used not only in chemical and medical industries but also in food and basic biological science .In this study an attempt was made for the screening and isolation of Keratinase producing bacteria from poultry waste.

Materials and methods

Collection and isolation of sample

Samples were collected from dump yards of poultry wastes at Solddevanahalli, Chikkabanavara, Devasandra, K.R.puram, Tannary road and Yashwanthpur in and around Bangalore, Karnataka, India. The samples were labeled after collected. These were spread onto isolation media Feather powder agar and incubated at 37^{o}C for 48 hours after serial dilution of 10^{-1} to $10^{-6.}$ See table -1:Tabulation for Samples Description

Screening of Keratinase production by plate assay

The isolates were screened for Keratinase activity. This was done by inoculating the organisms on the feather powder agar plates containing0.4 % feather powder (Washed feathers were dried at 50^{o}C in a forced draught oven (Gallenkamp, Ltd UK). The dried feathers were ground into fine fractions (<90, 90, 150, 300, 425 and 850μM) with test sieves of appropriate diameters) incubated at 37^{o}C for 48 hours. A clear zone around the growth of the bacteria was indicated to Keratinase activity see table -2:Tabulation for results of colony characteristics which shows Keratinase activity and Figure 1 for keratinase production

Identification of Bacteria

The isolated bacteria were identified based on cellular morphology, growth condition, grams staining, endo spore staining, capsule staining and biochemical tests (Pokorny, M., LJ. Vitale;1979). see table -3: Tabulation for results of Staining Techniques. And table -4: Tabulation for results of Various Biochemical tests

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Results and Discussion

Six bacterial isolates were obtained(Table:3) from soil samples of AP 1 to AP 10 (Table:1)and identified as *Bacillus cerus, Bacillus licheniformis* and *Staphylococcus aureus*. Morphologically and biochemically. The colonies were subjected to grams staining,capsule staining and endospore staining.The colonies which were positive and negative for Grams staining,Capsule and endospore staining were considered for further studies(Table 3&4).The selected colonies were streaked on feather powder agar plates.The plates were subjected to incubation for a period of 48 hours at 37^{o}C.The plates which showed clear zone around the streaked area of test organism was selected as Keratinase producing strain.The organisms named(Table2) showed the inhibition zone and was subjected to various biochemical tests(Table4). G isolates (Table2) showed the following results for the biochemical tests.These were positive for Methyl red test,Starch hydrolysis,Citrate utilization test.Oxidase test,gelatin hydrolysis test.urease test and nitrate reduction test ,and few isolates were shows negative for Voges Paskauer test,Indole test and Catalase test.After biochemical tests these organisms were confirmed to belong to the Bacillus and Cocci species (*Bacillus cerus* , *Bacillus licheniformis* and *Staphylococcus aureus*)producing protease.

Conclusion

The search for promising strains of Keratinase producers is a continuous process. The isolates which shows higher Keratinase activity were selected for biochemical characterization and identification. The organisms were identified as *Bacillus cerus* ,*Bacillus licheniformis* and *Staphylococcus aureus*. on the basis of data obtained in the present work it can be concluded that Bacillus and cocci species isolates can be employed in the production of Keratinase

Table -1: Tabulation for Samples Description

S.NO	DESIGNATION OF SAMPLE	SAMPLE COLLECTED AREA	SAMPLE COLLECTED LAND MARK	SAMPLE NATURE	SAMPLE PH
1	AP-1	Shivaji Nagar	Opposite to Maszid at Chiken center	Semisolid sticky Seems to Brown in colour	7.64
2	AP -2	Tannery Road	Near to Bus stop at Chiken center	Semisolid Seems to Black in colour	7.60
3	AP -3	Tannery Road	Near to Bus stop Chiken Center	Semisolid Seems to Brown in colour	7.72
4	AP -4	Tannery Road	Slaughter house opposite canal	Hard consist of sand and clay seems to Brown in colour	7.65
5	AP -5	Solddevanahalli	Near to Bus stop Chiken Center	Semisolid Seems to Brown in colour	7.62
6	AP -6	Chikka Banavara	Near to Bus stop Chiken Center	Sticky consist of sand and clay seems to Brick red in colour	7.44
7	AP -7	K.R.Puram	Devasandra lake Chiken dump	Semisolid Seems to red in colour	7.71
8	AP -8	Tin Factory	Opposite to Maszid at Chiken center	Semisolid Seems to red in colour	7.60
9	AP -9	Tin Factory	Near to Bus stop Chiken Center	Hard consist of sand and clay seems to Black in colour	7.26
10	AP -10	Yashwanth Pura	Fish market Near to Railway station	Sticky consist of sand and clay seems to Black	7.34

Table -2:Tabulation for results of colony characteristics which shows Keratinase activity.

STRAIN NO.	COLONY SURFACE	COLONY COLOUR	VISUAL CHARACTERISTICS	SHAPE OF THE COLONY	HEIGHT OF THE COLONY	PROTEASE/ GELATINASE ACTIVITY
G-1	Smooth	Brown	Opaque	Irregular	Raised	Positive
G-2	Smooth	Off white	Translucent	Circular	Raised	Positive
G-3	Smooth	Brown	Translucent	Irregular	Flat	Positive
G-4	Smooth	Off white	Opaque	Irregular	Raised	Positive
G-5	Smooth	Brown	Opaque	Irregular	Raised	Positive
G-6	Smooth	Brown	Translucent	Irregular	Flat	Positive

Table -3: Tabulation for results of Staining Techniques

STRAIN NO.	GRAM STAINING	MORPHOLOGY (BACILUS/COCCI)	ENDOSPORE STAINING	CAPSULE STAINING
G-1	Positive	Rods	Positive	Positive
G-2	Positive	Rods	Positive	Positive
G-3	Positive	Cocci	Positive	Positive
G-4	Positive	Rods	Positive	Positive
G-5	Positive	Rods	Positive	Positive
G-6	Positive	Cocci	Positive	Positive

Table -4: Tabulation for results of Various Biochemical tests

S.No.	SAMPLES	INDOLE	MR	VP	AMYLASE	NITRATE	OXIDASE	CATALASE	UREASE	GELATINASE	CASEIN / KERATINASE
1	**G-1**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**
2	**G-2**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**
3	**G-3**	**-Ve**	**+Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**
4	**G-4**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**
5	**G-5**	**+Ve**	**-Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**
6	**G-6**	**-Ve**	**+Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**+Ve**

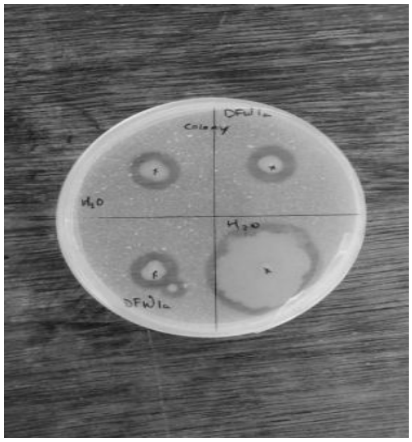

Fig. 1: Keratinase production

Cite this article"

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