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Isolation and characterization of keratinolytic Bacteria from poultry waste dumping soil

R. Manjula[1], S. Mary Shibiya[2], Hamida Shirin[3], Rekha. V[4]
School of Bioscience and Technology, VIT University, Vellore – 632014, Tamil Nadu, India

Abstract: The aim of the current study was to isolate, identify and characterize keratinolytic bacteria from poultry waste dumping soil areas in Vellore, TN, India. Keratinolytic bacteria are commonly found in poultry soils. It has an ability to produce the enzyme keratinase. The organism is selected based on the colony morphology and plated on selective medium (Horikoshi). Four bacteria (A, B, C, D) were isolated from collected soil sample .All isolates were screened for Keratinolytic activity by casein agar plate method and the protein estimation and protease assay was performed.

Keywords: Keratinolytic, Bacillus sp, Serial dilution, Protease assay.

I. Introduction

Keratin is a stable protein, it present in hair, nails, feathers etc. Due to the presence of stable protein it is used in feed supplement. The main disadvantage of keratin is degradation. The degradation capacity of keratin is low, it is the major problem in recycling. The keratin is degraded by the extracellular enzyme Keratinase. Degradation of keratin is taken place by attacking the disulfide bridge [Samuel etal,. H Prasad etal.] some organisms has the ability to produce the enzyme keratin such as Bacillus sp,. B. licheniformis, B. pumilus sps, Thermoanaerobacter, Actinobacteria, Vibrio Sp strain Kr2, Streptomyces albus, S. pactum, Fungi such as Dermatophilic and Saprophytic fungi, Aspergillus sp,. Trichophyton mentagrophytes, T. gallinae, T.rubrum, Microsporum canis, M. gypseum, Rhizomucor [Bo Xu. Qiaofang et al,.] Keratinize has a major role in the field of biotechnology. It involves in the degradation of waste from poultry and leather industry.

In the present study we focused on the isolation and characterization of Keratinase producing bacteria from poultry waste dumping areas in Vellore, TN, India.

II. Materials and Methods

The soil samples were collected from poultry waste dumping areas in Vellore (Katpadi) Tamil Nadu during August 2013. Soil sample were collected and transferred to sterile plastic bags. The samples were brought to Microbiology Research Lab VIT University, Vellore, TN, India.

A. Isolation of bacteria

The isolation of bacteria was performed by serial dilution and spread plate method on basal medium (nutrient agar). In 10ml of sterile distill water 1g of soil sample was taken in master dilution. Serial dilution procedure was carried out from 10^{-1} to 10^{-8}. 0.1 ml of the sample was taken from each dilution and plated on the nutrient agar by using spreader L-rod. The plates were incubated at 24-48hours at 37^0c.

B. Characterization and Identification of Keratinolytic Bacteria

Cultural Characterization

The test organisms were observed under the microscope, they are differentiated based on the colony morphology, size, shape, color, and nature of colony. [C M Williams etal,. Veslava Matikericiene etal,.]

Microscopic observation

The isolates such as A, B, C and D were Gram stained and observed under light microscope. The motility was tested by hanging drop method. [Zaqhloul TI etal, Z Jahan etal,.]

C. Biochemical Characterization

The biochemical test such as indole , methyl red, vogues proskauer , simmon citrate, catalase; oxidase, urease, nitrate reduction, gelatin hydrolysis, starch hydrolysis is carried out for the isolates and the results are tabulated. [Areeb Inamdar etal,. C M Williams etal,.]

D. Screening of Keratinolytic Bacteria

The bacterial isolates were inoculated in the Horikoshi medium incorporated with chicken feather. The P^H of the medium was adjusted to 8. The medium was incubated in rotary shaker at a speed of 150 rpm for 37^0c for 7days. After incubation the medium is centrifuged at 10,000 rpm for 10 minutes and the supernatant was tested for the enzyme activity. [D J Mukesh Kumar etal,.]

E. Enzyme Activity

The casein agar plate were prepared wells were made in the agar surface by using sterile well puncher and various concentration (such as 10µl, 20µl) of cell free supernatant was transferred into the well using a micropipette. The plate was incubated for 24 - 48 at 37°C. The plates were observed for zone of hydrolysis. [D J Mukesh Kumar etal,.]

F. Effect of pH

Horikoshi broth (containing 0.1% feather) was prepared and the pH adjusted to 3, 5, 7, and 9. And the test isolates was inoculated into medium. The inoculated broth was incubated at 37^0C for 48 hours.By using spectrophotometer the absorbence was measured at 590nm. [Areeb Inamdar etal,. E Vijay Kumar etal,.]

G. Effect of Temperature

Horikoshi broth (containing 0.1% feather) was prepared and bacterial isolates were inoculated. And the test isolates are incubated at different temperature 4, 25, 35 and 45°C. By using spectrophotometer the absorbance was measured at 590 nm. [Areeb Inamdar etal,. E Vijay Kumar etal,.]

H. Estimation of Protein by Lowry's Method

Folin-ciocalteu's method was used for the estimation of protein. Different concentration of BSA standard was prepared. All solution was made up to 1 ml with distilled water. Followed by 5 ml of alkaline copper sulfate and 0.5 ml of Folin-ciocalteu's reagent is used. Test sample was treated same manner without distilled water. The absorbance of the medium is measured at 600 nm against blank by using Bio-Rad.

I. Protease Assay

For the assay of protease, casein was used as a substrate. Various concentration of test samples (0.5, 1, and 1.5ml) with 5ml of TCA (Trichloroacetic acid) and 5 ml of sodium carbonate and followed by 1 ml of Folin-ciocalteu's reagent was added. The absorbance was measured at 600 nm by using Bio-Rad against blank.

Result and Discussion

In the present study four bacteria were isolated named A, B, C and D. The result for identification and characterization of all isolated organisms are reported in Table 1 and 2. All the isolates (A-D) were screened for keratinolytic activity on casein agar plate medium. The isolates which as the ability to produce the enzyme keratin shows the zone of hydrolysis. Among all, only the isolate D showed the zone of hydrolysis. The keratinolytic activity of isolates D showed in Fig.1

Isolates	Colony Morphology	Microscopic Characters		
		Spore staining	Gram staining	Motility
A	Rough	Non spore forming	Negative rods	Non motile
B	Yellow	Non spore forming	Negative rods	Non motile
C	Creamy	Non spore forming	Negative rods	Non motile
D	White mucoid	Spore forming	Negative rods	Motile

Table. 1. Microscopic Observation of Keratinolytic Bacteria.

The colony morphology of D organisms is showed in Fig 2 . The colonies of D isolates were found as white mucoid (Rhizoidal growth) gram negative spore forming motile colonies. Based on the cultural and biochemical pathway the isolate D is suggests as Bacillus sp. [Subhasish Saha etal.] The result for pH is reported in Table.3 and Fig.3. And temperature in Table.4 and Fig.4.The isolated organism D showed maximum enzyme activity at pH 7 and 35ºc is the optimum temperature for the enzyme activity.

Isoltes	**Indol**	**Methyl red**	**Voges proskauer**	**Citrate utilization**	**Catalase**	**Oxidize**	**Urease**	**Nitrate reduction**	**Gelatin hydrolysis**	**Starch hydrolysis**
A	-	+	-	+	+	-	+	+	-	+
B	+	+	-	+	-	+	+	+	-	+
C	-	+	-	+	-	+	+	-	+	+
D	-	-	+	+	+	-	+	+	+	+

Table. 2. Biochemical Characterization of Keratinolytic Bacteria

Table.3 Keratinolytic activity at Different concentration

Concentration (µl)	Zone of hydrolysis (mm)
10	**22**
20	**26**

Fig.1 Zone of inhibition on Casein agar plate

Fig.2. Morphology of Bacillus sp on Horikoshi medium

Table 4. Effect of different pH

Bacterial Isolate D (Bacillus sp)				
pH Range	3	5	7	9
OD at 590 nm	0	0.015	0.098	0.067

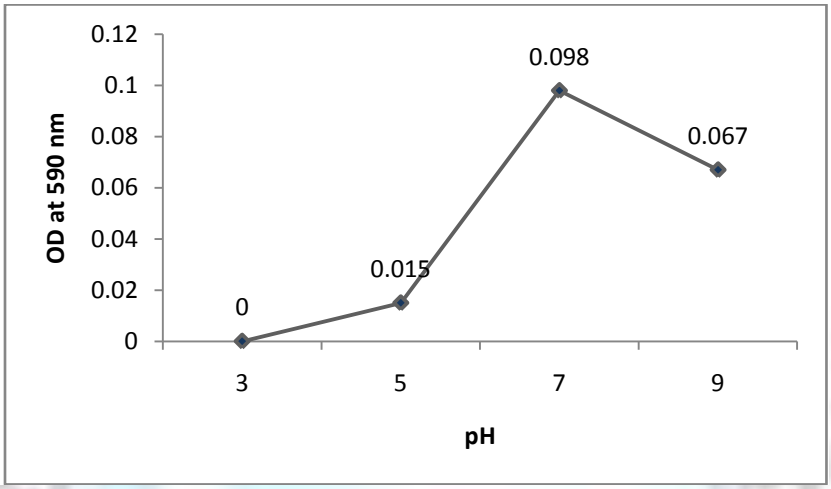


Fig.3: Effect of pH

Table.5: Effect of different Temperature

Bacterial Isolate B4(Bacillus sp)				
Temperature	4 ºC	25 ºC	35 ºC	45 ºC
OD at 590 nm	0	0.196	0.98	0.364

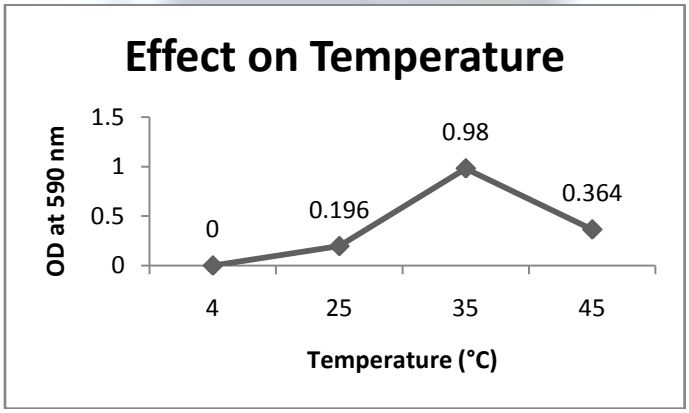
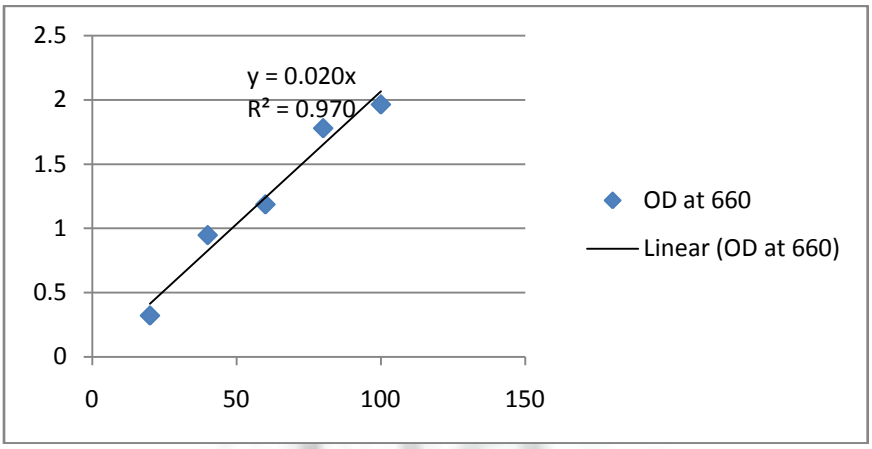


Fig.4: Effect of Temperature.

Estimation of protein by lowry's method Concentration 1µg/ml

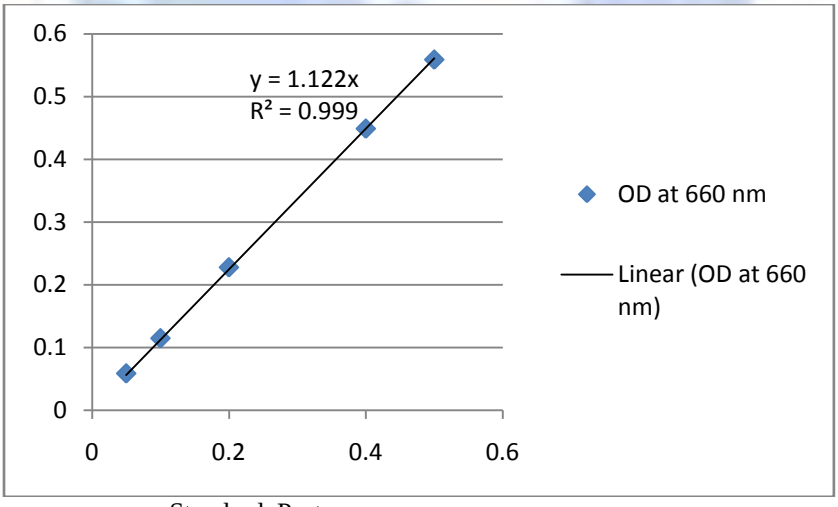


The concentration of protein in the test sample was found to be 0.882µg/ml. Various concentration of test samples were used (such as 0.5ml, 1ml, 1.5ml).

Enzyme activity is calculated and tabulated.

concentration of test sample (ml)	Concentration of tyrosine released	Enzyme Units/ml
0.5	1.1885	4.3578
1	0.9928	5.460
1.5	0.8137	8.9507

The figure showing Protease assay at different concentration.



Standard Protease assay

Conclusion

The isolated test organisms are screened for the keratinolytic and protease activity. Among all, the isolates D showed best enzyme activity in pH 7 and temperature 35^0C. In our present study we conclude that our test isolates D (Bacillus sp).It has a wider application in the field of biodegradation and to control the pollution and it is an ecofriendly method.

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