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Keratinolytic bacteria isolated from chick fecal waste

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ABSTRACT

The aim of this work to characterize keratinolytic bacteria isolated from chick fecal waste. Ten isolates were selected after growth on solid medium with feather meal as carbon and nitrogen source. They were screened for keratinolytic activity on milk Agar plates. All the ten isolates were gram positive (*Bacillus Sp*). The bacteria grew on wastes of keratin such as, raw feather, Feather meal. Keratinolytic activity was determined during growth, proteolytic character of crude enzyme was assessed using keratin azure as substrate. The keratinase was highly active on the substrate. The keratinolytic isolates have potential biotechnological use in process involving keratin hydrolysis. © 2010 Trade Science Inc. - INDIA

KEYWORDS

Feather meal;
Keratinase;
Proteolytic;
Keratin azure.

INTRODUCTION

Feathers are composed of over 90% of keratin and produced in a huge amount as a waste by poultry farms worldwide. Accumulation of feathers will cause major environmental pollution and feather keratin wastage^[6]. Keratins which are among the hardest to degrade animal proteins. They are major components in poultry feather processing units. Keratinolytic enzymes may have potential role in biological industrial processes that involving keratin containing wastes from the poultry and leather industries^[3]. Feathers contain huge amounts of useful protein and amino acids they have used to increase the fish weight in the pond due to the consumption of amino acids. Bacterial keratinases are specific interest because of their action on insoluble keratin substrates^[8].

The feather wastes were used to produce feather meal through heat process. It is a nutritional value pro-

duce in low amount^[18]. Cultivation situations are important for success degradation of feathers and enzyme production.

The *Bacillus sp* was isolated from chick fecal waste

TABLE 1 : Effect of temperature on growth a keratiolytic activity of bacteria isolated from chick fecal waste

Isolates	50°C	60°C	70°C	80°C
krb 1	+	+	-	-
krb 2	+	+	+	+
krb 3	+	+	-	-
krb 4	+	+	+	+
krb 5	-	+	+	-
krb 6	+	+	+	+
krb 7	-	+	-	-
krb 8	+	+	-	-
krb 9	+	+	-	-
krb 10	+	-	-	-

+Growth with clearing zones were observed. -No growth or clearing zones were observed

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TABLE 2 : Keratinase production on feather meal with different time intervals

Isolates	Keratinase activity (u/ml)			
	12 h	24 h	36 h	48 h
Krb 2	180	248	250	270
Krb 4	210	320	320	325
Krb 6	280	410	415	421

TABLE 3 : Protein production on feather meal with different time intervals

Isolates	Protein content (mg/ml)			
	12 h	24 h	36 h	48 h
Krb 2	0.85	1.21	1.42	1.68
Krb 4	1.3	1.42	1.82	1.91
Krb 6	1.28	1.48	1.62	1.90

and was capable to completely degrade chicken feathers.

In this study, we describe the isolation and characterization of thermophilic bacteria shows keratinolytic activity isolated from a poultry unit at Namakkal, Tamilnadu, India.

MATERIALS AND METHODS

Microorganisms isolation

Chicken fecal waste was collected from local poultry farm. The samples were mixed in 0.85% saline solution, make suspension up to 10^{-8} and used to streak milk agar plates. Composition of milk agar (g^{-1}) 5g peptone, 3g malt extract, 100ml milk and 12g agar. Which were incubated at $37^{\circ}C$ for 24hrs for primary screening test. The bacteria produced clearing zones in the milk agar plates were selected^[17].

The bacterium was identified as *Bacillus Sp* according to Berge's manual classification.

Bacillus, Bacterial strains were designated as krb1 to krb 10 were isolated from chicken fecal waste. A previously characterized *Bacillus sp*. Strain krb 5 that presented keratinolytic activity. Bacterial identification was performed on colony morphology, gram staining technique, and biochemical tests. Comparing the report with standard species.

Effect of temperature on growth and proteolytic activity

Milk agar plates (g^{-1}) 5g peptone, 3g yeast extract, 100ml sterile non-fat milk and 12g agar [Riffle et al.,]

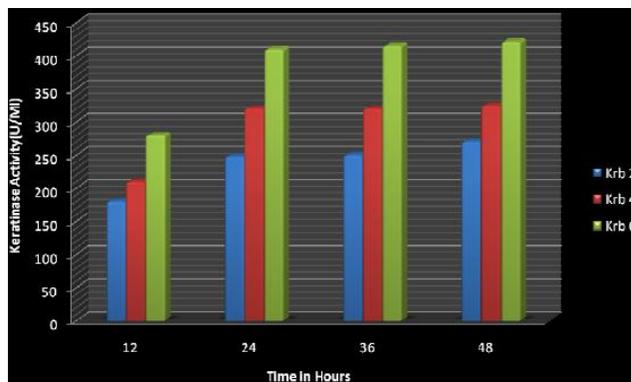


Figure 1 : Assay of keratinase activity

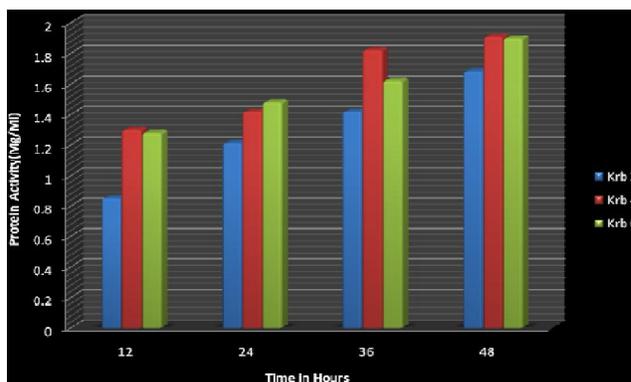


Figure 2 : Assay of protein activity

were prepared. Bacteria were incubated at $50^{\circ}C$, $60^{\circ}C$, $70^{\circ}C$, and $80^{\circ}C$ for 12h. Bacterial Strains were produced clearing zones in the milk agar medium were selected.

Enzyme assay

Keratinolytic activity was assayed with keratin azure as substrate. The reaction mixture contained 1ml of keratin azure suspension and 1ml appropriately diluted enzyme.

The reactions were carried out at $50^{\circ}C$ constant agitation of 200r/min for 1 hr. After incubation, the reactions were stopped by adding 2ml of 0.4mol/L TCA and followed by membrane filtration to remove the substrate.

The filtrate was measured colorimetric ally at 595nm.

Protein assay

Protein concentration was determined by Lowry et al. method. Egg albumin used as a standard.

RESULTS AND DISCUSSION

Ten isolates were selected from poultry unit fecal

waste was able to grow on medium containing feather meal as carbon & nitrogen source. After being incubated 24 hr, a plate containing milk agar showed the growth of several colonies. The strains produced clearing zones in milk agar plates characterized by proteolytic activity.

The largest clearing zone was observed for isolates krb2, krb4, and krb6. These strains were selected & used for keratinolytic activity assay.

DISCUSSION

Bacillus sp were isolated from poultry unit that have keratinolytic and feather degrading ability of the keratin wastes. The *Bacillus sp* has different characteristics, such as a broad temperature range of growth. The optimal proteolytic activities were identified between 50 to 80°C.

Bacteria growing on medium containing feather meal as a carbon and nitrogen source presented variable activity on keratin azure, suggesting that this enzyme may be inductive. Substrate level in the medium may regulate enzyme production. Strain krb 2, krb4, and krb6 showed to be more adapted to keratinase production using keratin as substrate. Since the maximum keratinase activity of the isolates were observed during early growth.

The keratin azure hydrolysis ratio was higher for strains krb2 krb4 and krb6 suggesting preferred utilization of keratin as substrate. Isolation of keratinolytic Bacterium considering that feather protein has been showed to be an excellent source of metabolizes protein. The keratinolytic strains could be used to produce

fish feed protein the selected isolates were able to grow and show keratinolytic activity.

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