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RESEARCH ARTICLE

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PRODUCTION OF KERATINOLYTIC ENZYME BY A NEWLY ISOLATED FEATHER DEGRADING *BACILLUS SP*. FROM CHICK FEATHER WASTE

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ABSTRACT

A novel feather-degrading *Bacillus Sp*. was isolated from chick manure. The bacterium produces keratinolytic enzyme using chicken feather as a sole of carbon and nitrogen source. The effect of temperature, pH on keratinase production of this keratinolytic strain was studied. Maximum enzyme activities were obtained within 48 hrs (780U/ml) of cultivation. The optimal conditions for the keratinolytic activity were determined to be pH 9.0 and temperature 60°C; however the enzyme showed a broad range of pH between 5-10 and temperature 20°C - 100°C. In addition, the *Bacillus sp* was able to completely degrade within a period of 48 hrs.

KEYWORDS

Feather, Keratinase, Degradation, Environment, Protein

INTRODCTION

Keratinases (E.C. 3.4.99.11) belong to the group of serine hydrolases that are capable of degrading keratin, a fibrous and insoluble structural protein extensively cross-linked with disulfide, hydrogen and hydrophobic bonds (Anbu et al., 2006.,) Keratins, which are among the hardest-to-degrade animal proteins, are the major component proteins in poultry feathers and are characterized by a tightly packed form in α- helixes and β sheets with a high degree of disulfide bonds (Yasushi shigeri et al., 2009).

Keratinase is an extra cellular enzyme used for the bio degradation of keratin. Keratinase is produced only in the presence of keratin substrate. Keratinase attacks the disulfide bond of keratin to degrade it. Some microbes have been reported to produce keratinase in the presence of keratin substrate. Keratinase producing microorganisms have ability to degrade chicken feathers, hair, nails, wool etc. (Gradišar et al 2005, Cai et al 2008)

Bacterial strains are known which are capable of degrading feathers. These bacterial strains produce enzymes which selectively degrade the beta-keratin present 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 betakeratin.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. Keratinase producing microorganisms have the important industrial application in fermentation technology. Submerged fermentation of poultry waste by microorganism producing keratinase helps in the conversion of nonsoluble keratin (feather) into soluble protein (Suntornsuk et al.,)

Microbial keratinase has become biotechnologically important since they target the hydrolysis of highly rigid, strongly cross-linked structural polypeptide keratin recalcitrant to commonly known proteolytic enzymes : trypsin ,pepsin and papain. These enzymes are largely produced in the presence of keratinous substrates in the form of hair, feather, wool, nail horn etc.during their degradation.

The promising applications of keratinolytic proteases include enzymatic dehairing of leather,detergent industry and development of biodegradable films (Jin-Ha Jeong *et al.,* 2010).

Amongest the industrially important enzymes, microbial keratinases are stimulating tremendous interests in the enzyme market owing to the fact that there is a great demand for developing biotechnological alternatives for recycling of keratin wastes, converting unused chicken feather to useful value added products (Williams et al., 1990)

In the current study we have focused on the isolation and characterization of extracellular keraitnase producing *Bacillus Sp*. from the chick feather waste of feather processing units at Namakkal, Tamilnadu, India.

MATERIALS AND METHODS

Isolation of keratinolytic microorganism

Chick manure was collected from a local poultry industry. The sample was flooded in

saline solution 0.85%, suspension up to 10^{-5} were made and used to streak feather meal agar plates (10 gl^{-1} feather meal; 0.5 gl^{-1} NaCl; 0.3 gl^{-1} K_2HPO_4; 0.4 gl^{-1} KH_2PO_4; 0.1 gl^{-1} $MgCl\text{-}6H_2O$; 0.1 gl^{-1} yeast extract; 15 gl_{-1} agar, pH 7.5) which were incubated at 37°C for 24 hrs.(Daniel *et al.,*2008).

Screening on Skim milk agar plates:

Skim milk agar (Himedia) was prepared and the above dilutions were streaked on milk agar plates for testing the caseinolytic activity of the organism. Bacteria were inoculated onto plates and incubated at 37°C for 24 h. Strains that produced clearing zones in this medium were selected (Zerdani *et al*., 2004).

Subculturing:

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 studies of isolated bacterial strains:

Bacterial identification was conducted on morphological, physiological and biochemical tests. Results were compared with *Bergey's Manual of Determinative Bacteriology*, 8th edition (Buchanan and Gibbons, 1974). The strain was also identified by chromogenic method on the bacillus differential agar from Himedia M1651 (India) recommended for rapid identification of *Bacillus* species from a mixed culture. The medium contains peptic digest of animal tissues and meat extract, which provide nitrogenous compounds. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by the pH indicator phenol red (Sarita Agrahari et al., 2010).

Assay of Keratinase activity:

Keratinase activity was assayed by the modified method of Yu et al (1968). 20 mg of Chicken feather powder was suspended in3.8 ml of 100mM Tris-HCl buffer (pH 7.8) to which 0.2 ml of the enzyme sample was added. The reaction mixture was incubated at 37°C for 1 hour. After incubation the assay mixture was cooled in ice cold water for 10 min and the debris were removed by filtration through a Whatman no.42. The filtrate was subjected to filtration through a Millipore cellulose filter (0.45µm) under vaccum. The absorbance of the mixture was measured at 280 nm (Anbu *et al.,* 2007).

Assay for proteolytic activity

The protease activity was determined using azo-caesin For this assay, a reaction mixture containing 0.3 ml of 0.5% azo-caesin in sodium acetate buffer (pH 5), 0.1 ml of 10 mM $CaCl_2$, 0.2 ml of enzyme solution and 0.4 ml of sodium acetate buffer (pH 5) was incubated at 37°C for 30 min. The reaction was then terminated with 20% trichloroacetic acid. The mixture was centrifuged at 10,000 rpm for 5 min and the filtrate was neutralized with 1.8 N NaOH and absorbance was read at 425nm. One unit (1 U) of protease activity is defined as the amount of enzyme that produced an increase in absorbance of 0.5 under the assay conditions (Sangeli et al., 2000).

Protein determination:

Protein concentration of the enzyme preparations were determined according to the method described by Bradford (1976), using bovine serum albumin as the standard.

Determination of bacterial growth and feather Degradation:

Bacterial growth was determined by total plate count on nutrient agar. Feather in culture medium was harvested by filtration through Whatman Number 3 filter paper, washed twice with distilled water and dried at 105°C to constant weight. The percentage of feather degradation was calculated from the differences in residual feather dry weight between a control and treated sample(Geun-Tae park *et al.,* 2009).

RESULTS & DISCUSSION

Isolation, characterization of keratinolytic strains:

The bactertia was found that the enriched feather degrading culture contained micro-organism exhibited keratinolytic activity. The feathers were fully solubilized within 48h of incubation with the microbes from selected soil. The bacterial strain that visually degraded feather was isolated and allowed to grow on medium containing feather meal powder as sole carbon and nitrogen source. The selected strain produced clear zones on incubation at 30°C for 72 h suggesting the presence of keratinolytic activity (Fig. 1).

Figure 1

The identification of the keratinolytic bacteria was based on cell morphology, colony morphology, and several other methods. These results suggested that the strain belong to genus *Bacillus.* (Sarita Agrahari *et al.,* 2010).

The newly isolated *Bacillus sp.* is a novel strain which can degrade feather keratin. Compared to most other keratin-degrading strains, it can degrade the native feather in much shorter time (48 h) and has a relatively high keratinase activity (780U/ml) (Figure 2)**.** Partial degradation was observed after incubation at 60°C for 48 hrs. Williams et al., (1990) reported that *Bacillus licheniformis* PWD-1 degraded chicken feather completely at 50°C in 10 days. Bockle et al.(1995) demonstrated that *Streptomyces pactum* DSM40530 partially degraded native chicken feather at 50°C**,** the maximum feather degrading activity at 50°C. We focused that feather degrading activity of *Bacillus sp*. may be an interesting property for biotechnological industries. The maximum activity of partially purified enzyme at the 60% fraction with the high yield of 98.2% and specific activity 0f 5.3 was shown in table 1.

The isolated bacterium showed high keratinolytic activity when cultured on feather medium. Optimum keratinolytic activity was observed at 60°C and pH 9.0 as shown in Figure 2 and 3. The enzyme also showed to be stable at 60°C and pH 9.0 (Fuhong Xie *et al.,* 2010).

The optimum temperature of keratinases ranging from 30 to 80°C. Nam *et al.,* (2002) showed exceptionally high temperature optima of 90 and 100°C, respectively, with a half-life of 30 and 90 min respectively. The pH and temperature stability indicates that keratinases are generally active and stable over a wide range of pH from 5 to 13 (Bockle *et al.,* 1995). *Bacillus* strains are ubiquitous microorganisms, which can grow on natural media without any special requirements. These properties can be exploited in the degrading feathers, which are produced in huge amounts throughout the world.

Moreover, *Bacillus* strains are thermophile microorganisms and this property can be used in controlled process for efficient and fast degeneration of feathers. This novel keratinolytic isolate could be a potential candidate for degradation and utilization of feather keratin.

Table 1
Enzyme activity of partially purified enzyme

	Total activity(U/ml)	Total protein(mg)	Specific activity(u/mg)	% yield	Purification fold
Supernatant	5040	282	17.9	100	1.0
Ammonium sulphate fractionization(40-60%)	2553	26.3	97.1	50.7	5.4

Figure 2

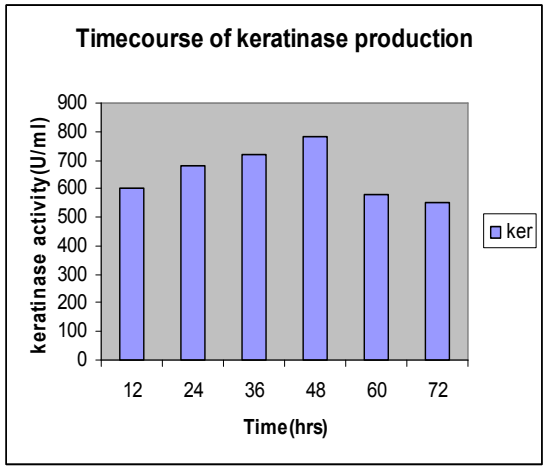


Figure 3

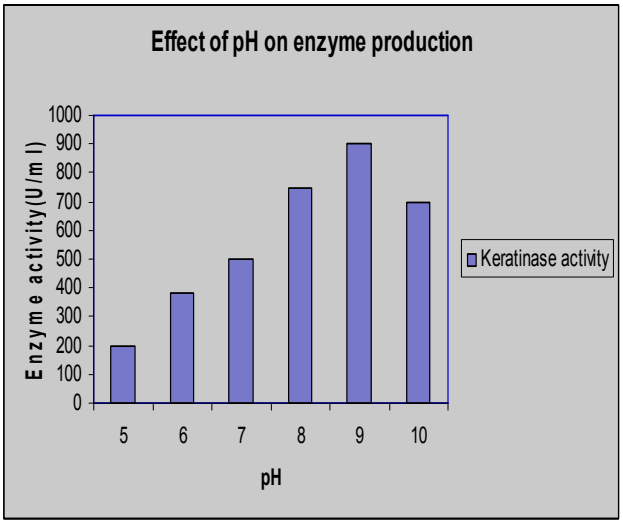
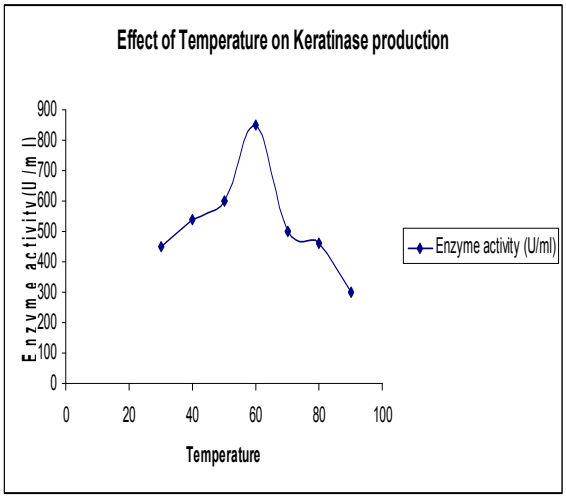


Figure 4



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