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KERATINOLYTIC PROTEASE PRODUCTION FROM KERATINACEOUS WASTES

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ABSTRACT

Keratin waste is generated in large amounts from various industrious as well as a by-product of commercial poultry and leather processing units and is highly resistant to degradation by conventional proteolytic enzymes viz. Pepsin, Papain and Subtilisin. Keratinases are a particular class of proteolytic enzymes that display the capability of degrading insoluble keratin substrates by diverse microbes. In the present study we have tested the keratinolytic ability of our lab isolate *Bacillus sp. Ker17* strain by using four different keratin wastes under submerged fermentation at 37 °C temperature and pH 9.0 Among different keratins added to basal medium, nails were effectively degraded about 97.9(±0.2) % within about 5.3 (±0.2) days where soluble protein produced was 37.7 ±2.0 mg/ml and protease activity exhibited at 343.1 ±0.1 U/ml. However, different keratin wastes degradation time varied from 5.3 to12.5 days. Ker17 strain can be an effective tool for the processing and or management of solid keratin litter and gives a remedy for environmental concern over abundant keratinaceous waste conversion to value added product formation in biotech industry with its promising keratinolytic abilities.

KEY WORDS keratin wastes, protease production**,** *Bacillus species Ker17*, keratin utilization

INTRODUCTION

Abundant amount of keratinaceous waste materials are readily available in nature as feather, hair, wool, horns, nails and hoof etc but have limited applications and usage [1]. Due to high degree of cross-linking by disulfide bonds and hydrophobic interactions, they are insoluble and resistant to degradation by common proteolytic enzymes such as trypsin, pepsin, and papain. Secondary confirmation allowed keratins to be classified as α (α-helix of hair and wool) and β (β-sheets of feather) keratins [2]. Therefore, these enzymes could be used after chemical pretreatment or steam cooking of the feather wastes; where in the processes utilize significant energy and also destroy some of the amino acids [3]. In mature chicken, feather accounts up to 5–7% of the live weight. Worldwide, several million tons of feathers are generated annually as waste by poultry-processing industries. Considering its high protein content, this waste could have a great potential as a source of protein and amino acids for animal feed and for many other applications [4]. Keratinases (E.C 3.4.21/24/99.11) are a particular class of proteolytic enzymes that display the capability of degrading insoluble keratin substrates. These enzymes have gained importance in recent years, as several potential applications have been associated with the hydrolysis of keratinous substrates [5]. The development of enzymatic and/or microbiological methods for the hydrolysis of feather into soluble proteins and amino acids could be extremely attractive, as it may offer a cheap and easy method for the production of valuable products, such as rare amino acids like serine, cystein, and proline, or for the development of slow-release nitrogen fertilizers, glues, and biodegradable films [6]. Several feather-degrading bacteria have been isolated and characterized till date from soil, poultry wastes, hair debris and animal skin; most of them were confined to genera *Streptomyces* and *Bacillus* apart from various fungi [1]. Feather may also find important application in the fermentation industry for the production of commercial enzymes [4]. Hence there is scope for the utilization of these keratinaceous wastes for keratinolytic protease production by using microbial fermentation technology. Present study describes the evaluation of different keratinaceous wastes for the production of keratinolytic protease by a feather degrading *Bacillus sp. Ker 17* strain isolated previously from a local slaughterhouse soil.

MATERIALS AND METHODS

Keratin source: All keratin materials such as human hair and nails collected from a barber shop; buffalo horns collected from slaughter house, sheep wool collected from a local retailer. All were thoroughly washed with tap water followed by mild detergent wash, sun dried for 1 day and later soaked in chloroform: methanol to remove lipid content later washed with distilled water and sun dried for 12 hrs. Sheep wool and human hair were cut into 2-3 cm fragments. Horns and nails were grinded to fine powder and preserved in dry containers till use.

Table: 1 Table: 1 Effect of different keratin sources on protease production by *Bacillus sp. Ker17* strain

Keratins	Soluble protein (mg/ml; ±SD)	Protease activity (U/ml; ±SD)
Horns [a]	25.9 ±1.7	215.6 ±2.2
Hair [b]	41.5 ±3.1	185.8 ±5.0
Nails [c]	37.7 ±2.0	343.1 ±0.1
Sheep wool [d]	23.1 ±4.4	215.8 ±1.3

Results at varied degradation timings; a=9.3; b=6.9; c=5.3; d=12.5 days respectively

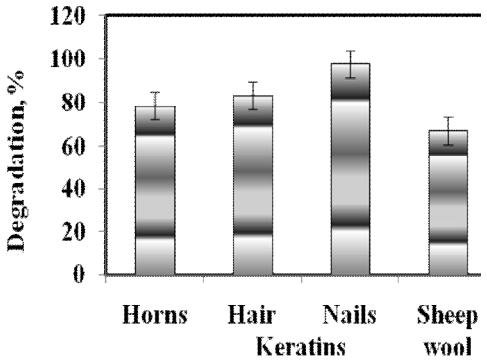

Fig. 1 Degradation pattern of different keratin sources by Bacillus sp. Ker 17

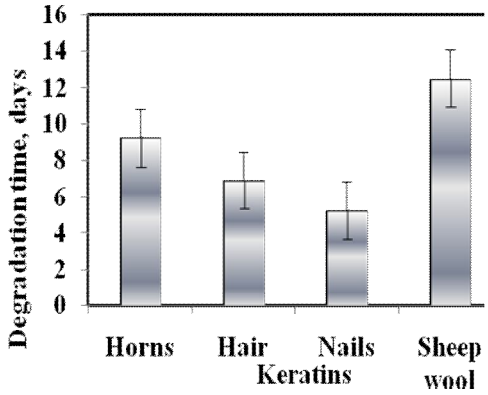

Fig. 2 Degradation time of different keratin sources by Bacillus sp. Ker 17

Chemicals: All the chemicals used in the study were of analytical grade from our laboratory unless and otherwise specified.

Test organism: Keratinolytic strain used in the present study was previously described *Bacillus sp. Ker 17* [7] from our laboratory. This strain was maintained on nutrient agar slants at 4°C refrigeration.

Medium and fermentation conditions

1% v/v inoculm (overnight grown culture; A_{660} ~0.6-1.0) was added to 50 ml basal mineral medium [7] in 250ml flasks supplemented with 1% each keratin waste in different flasks. Un inoculated flasks kept as control at each case. ***p***H of the medium was set to 9.0 by dilute NaOH or HCl prior to sterilization. Fermentation was carried at 37 °C and 160rpm agitation for 10-12 days. On visual basis, contents of the flasks were filtered through Whatman filter paper followed by centrifugation and supernatant was used for analytical procedures.

Determination of keratin wastes degradation percentage

Different keratin waste material degradation percentage was determined by weight loss method as per [8]. The percentage of degradation was calculated by weight loss from the difference in un-utilized keratin dry weight of control (without inoculation) and treated sample.

Protein determination

Soluble protein of fermented broth filtrate was measured as per Lowry et al 1951

Protease assay

Proteolytic activity was measured using casein as substrate. Reaction initiated by adding 100 µl crude enzyme to 900 µl 1 % casein (dissolved in 100 mM Tris-HCl at ***p***H 9.0) and incubated at 40 °C for 10 min. Reaction was terminated by equal volume chilled TCA (10 %) addition and left at room temperature for 30 min. Precipitated (residual) casein was removed by centrifugation at 5000 rpm for 10 min. Acid soluble amino acids released in to the

supernatant were measured by Lowry et al 1951 using Folin phenol reagent. Suitable blanks were prepared simultaneously. Protease activity expressed in U/ml

One unit of protease activity can be defined as the amount of enzyme that liberates 1 µg of tyrosine per minute under described assay conditions [7]. All the experiments were carried in duplicates. Mean value of two experiments ± standard deviation (SD).

RESULTS AND DISCUSSION

Keratin wastes degradation by Ker17 strain

The ability of Ker 17 strain to degrade and produce keratiolytic protease from various keratin sources such as horns, hair, nails and sheep wool was studied. Keratinolytic property of the strain was confirmed by different keratin wastes degradation pattern as shown in Fig.1. when they are added as only source of carbon and nitrogen. Of the different keratin wastes added to basal mineral medium at 1%w/v , nails were majorly degraded about 97.9(±0.2) % where as sheep wool stood at least by 67.2(±1.7) % utilization. Remaining keratins like horns, 78.7 (±1.0) % and hair 83.4(±1.8) % degraded by Ker17 strain. However, different keratin wastes degradation time varied from 5.3 to12.5 days. As illustrated in Fig.2, nails were taken short time for degradation about 5.3 (±0.2) days whereas sheep wool taken long time for degradation i.e. 12.5(±0.1) days and remaining keratin wastes such as horns 9.3(±0.1) and hair 6.9(±0.4) days taken for maximum degradation. Although all the flasks incubated beyond the aforesaid timings, there is no any improvement in degradation pattern with respect to increase in the time.

Keratinolytic protease productivity by Ker17 strain

As a result of degradation of different keratin wastes by Ker17 strain, secreted protease has shown activity from 215.6 ±1 to 343.1 ±0.1 U/ml. When nail used as keratin inducer maximum protease activity was recorded as 343.1 ±0.1 U/ml where soluble protein was found at 37.7 ±2.0 mg/ml. However, rest all keratins also produced protease as well as soluble protein as summarized in table 1.

Endo spore forming *Bacillus species* are known producers of keratinase enzyme along with other hydrolytic enzymes which degrades various forms of keratin such as hair, feathers, hoof, wool and nails etc [9.10]. Few gram negative bacteria reported *Chryseobacterium* [11], *Stenotrophomonas* [12]. Keratinases are largely produced in a basal medium with a keratinous substrate using keratin as the sole source of carbon and nitrogen [1] as it is obtained in the present study all the keratinaceous wastes induced extra cellular keratinoloytic protease production. Alkaline ***p***H from 6 to 9 supports keratinase production and feather degradation in most microorganisms. Alkaline ***p***H possibly favours keratin degradation and higher ***p***H modifies cystine residues to lathionine [13], thereby accessible for keratinase action. Similarly, increase in alkalinity usually accompanies keratinolysis [1] which is attributed to deamination reactions leading to the release of ammonium [14]. Most keratinases possess proteolytic activity; however, none of them possess the disulfide-reducing potential. Therefore, reduction in disulfide bonds is accomplished by di-sulfide reeducates sulfite elaboration or redox potential of cells [15]. Many authors have reported keratinolytic activities for some *Bacilli* as 51.7-67.9 U/ml [6]; 39.1 U/ml [16]. Most authors have reported complete or partial degradation of different keratin by diverse microbes in the range of 4 -16 days [1]. In the present study, all the four keratin substrates induced keratinase enzyme production and their structural complexity extended their degradation time by *Bacillus species Ker17* strain. This strain may be employed for effective keratin litter management and can be a valuable tool in biotech industry for value added products generation through its promising keratinolytic property.

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