Isolation of keratinase producing bacteria

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Abstract:

Keratin is insoluble structural protein present in nails hair, feather,wool and horns of animals. In the present study keratin degrading bacteria isolated from woollens dumped waste. Ten keratin degrading bacterial strains were isolated. Standard microbiological characteristics were studied. Strain KR1 tentatively identified as Bacillus sp. Keratinolyitc activity of crude enzyme determined .One of the isolate KR1showed highest specific keratinolytc activity 1.9 IU/mg. Optimum condition for keratin degradation were P^H 7.5,temperature 37^0 C and wool keratin concentration 3%. Microbial keratinases have found eco friendly way to treat keratin rich waste and transformation into valuable products.

Key words:

Keratinolytic bacteria , keratinase , Bacillus sp, optimization.

Introduction:

Keratin forms a major component of the epidermis and its appendages viz. hair, feathers , nails, horns, hoofs, scales and wool. On the basis of secondary structural confirmation , keratins have been classified into α (α -helix of hair and wool) and β (β-helix of feather) (Akhtar, 1997) Keratin is an insoluble protein macromolecule with very high stability and low degradation rate . Keratinase is an extracellular enzyme used for the biodegradation of keratin. Keratinase is produced only in the presence of keratin substrate. (A. A. Onifade, 1998). High protein content of keratin waste can be used as a good source of protein and amino acids by systemic recycling. Recycling of feather can provides a cheap and alternative protein feed stuff. Further this can be used for animals feed and for many other purposes. However, poor digestibility of keratin is a problem in recycling (H. Takami, 1992)Maximum amount of wool produced in India i.e. 85% is carpet grade. 10% wool produced in India is coarse grade. With 44% production of wool, Rajasthan leads all states in India. Rajasthan is followed by Jammu & Kashmir(13 percent), Karnataka (12 percent) Gujarat.

Disposal of wool scouring sludge is becoming more problematical, due to the need to satisfy environmental legislation concerning what is regarded as hazardous waste, and to the increasing cost of landfill and the scarcity of landfill sites. By 2010, therefore, landfill sites will only accept non-recoverable waste and inert waste, so that the disposal of wool scouring sludge to landfill will then be an untenable option. Bioremediation of keratin rich waste may produce a safe, saleable product and will be environment friendly method. Hence present study is aimed to isolate efficient keratin degrading microorganisms.

Material and methods:

1. Collection of Sample Black carpet wool pieces brought to laboratory in test tube. After surface repeated sterilization with mercuric chloride solution. It was suspended in sterile saline tube and stirred for few minutes. After centrifugation supernatant was used for further study.

2. Enrichment

The medium (XIANG LIN, 1992) slightly modified contained, per liter, the following:0.5 g of NH_4Cl, 0.5 g of NaCl , 0.3 g of K_2HPO4, 0.4 g of KH_2PO4, 0.1 g of $MgCl_2$ $6H_20$, 0.1 g of yeast extract, and 10 g of wool form black carpet. The pH was adjusted to 7.5. Wool was washed with sterile saline followed by alcohol 95% and dried . Medium was sterilized by autoclaving.

After sterilization enrichment medium was inoculated with 2 ml sample. Flask along with another uninoculated control flask incubated at 37° C on orbital shaking incubator (110 rpm) for 48 hrs.

3. Isolation of keratinolytic bacteria.

Keratin agar medium prepared by addition of 2.5% agar agar in to the above described medium .Medium was sterilized by autoclaving and plates were prepared. 0.1 ml enrichment culture sample inoculated on the plates of keratin agar by using spread plate technique. Plates were examined at an interval of 24 hr. incubation till colonies appear on keratin agar plates. Standard macroscopic and microscopic characters were determined. Cultures were purified and cultures maintained on slants for further study.

4. Characterization

Different biochemical characteristics of strain KR1 were determined such as sugar fermentation , IMViC test, catalase , oxidase,NO_3 reduction, H_2S production,gelatinase ,caseinase and amylase activity.

Enzyme Production : Keratin broth (500ml) was inoculated with 2% inoculum(0.3O.D.)

And incubated for 7 days on orbital shaking incubator (110rpm) at 37° C. After incubation for 7 days , broth was centrifuged 5000rpm for 10 minutes at 20°C. Supernatant was separated used to study ketatinolytic activity. Protein content was determined by using Lowery method using bovine serum albumin.

Keratinolytic activity- Keratinolytic activity assay was according to Yamamura et al. (2002) and Z.Fang et al(2013). Sample containing 1ml of 50mg of wool powder was mixed with 1ml enzyme solution and incubated at 50°C for 1 hr .The reaction was terminated with 2ml of 4% trichloroacetic acid (TCA).

200µl of supernatant was mixed with 1ml of Folin –Ciocalteu reagent and incubated at 30°C for 1hr. absorbance was measured at 600nm . Tyrosin standard curve used as reference to determine keratinolytic activity. 1 µmol tyrosine liberated per hr equivalent to one unit of keratinolytic activity.

Results and Discussion:

In the enrichment broth growth of keratinolytic bacteria observed in terms ofturbidity. Wool which was added in the flask was found to be degraded in small pieces. Inoculated Flasks were compared with uninoculated control flask. Wool was separated from one of the flask and thoroughly washed. After drying mass reduced due to keratinolytic activity was determined. There was 40% decrease in biomass. Specific keratinolytc activity 1.9 IU/mg for crude enzyme produced by keratinolytic strain KR1 .

On keratin agar colonies of keratinolytic bacteria were observed as shown in **figure1.** Standard microbiological characteristics were determined. One of the isolate showing efficient keratinolytic activity selected for further study .**Table 1** keratinolytic isolate strain KR1 is motile Gram positive non sporulating rod shaped bacteria. Biochemical characteristics of strain KR1 are recorded in **Table 2**, this strain showed strong proteolytic activity. Tests for caseinase, gelatinase and amylase were positive. From the present study isolate obtained possess keratinolytic activity and is useful for bioremediation and for production of valuable products.

Table 1: Macroscopic and microscopic characteristics of Keratinolytic strain KR1

Colony character	Observation
Size	1 mm
Clear zone	2mm present
Shape	Circular
Colour	White
Margin	Entire
Surface	Smooth
Opacity	Non opaque
Elevation	Elevated
Consistency	Butereous
Motility	Non Motile
Grams nature	Gram positive non sporulating rods

Table 2: Biochemical characteristics of Keratinolytic strain KR1

Biochemical Tests	Results
Indole test	Negative
Methyl Red	Positive
Voges proskauer	Negative
Citrate test	Positive
Catalase	Positive
Oxidase	Positive
Gelatinase	Positive
Cellulose hydrolysis	Positive
Starch hydrolysis	Positive
caseinase	Positive

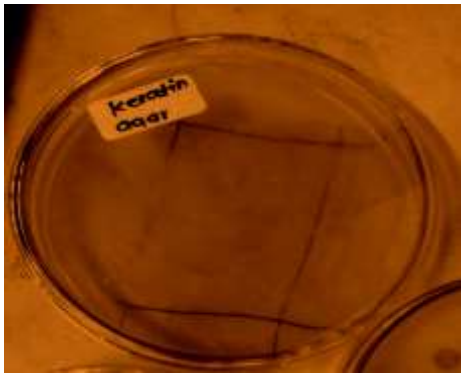

Figure1-Keratinase activity of strain KR1

Referances

A. A. Onifade, N. A.-S.-Z. (1998). Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes. *Biores Technol., 1998, ,* 66(1):1–11.

Akhtar, W. a. (1997). Fourier-transform Raman spectroscopy of mammalian and avian. *SpectrochimActa .,*, 53: 81-90.

H. Takami, N. S. (1992). Degradation of Human Hair by a ThermostableAlkaline Protease from Alkaliphilic . *Bioscience, Biotechnology, and Biochemistry*, 1347-6947.

Onifade AA, A.-S. N.-M.-Z. (1998). Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement. *Biores Technol .*, 66(1):1–11.

XIANG LIN, t. C.-G. (1992). Purification and Characterization of a Keratinase from a Feather-Degrading Bacillus licheniformis Strain. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY,*, 3271-3275.

Yamamura. S., M. Y. (2002). Keratin degradation:a cooperative action of two enzymes from Stenotrophomonas sp. . *Biochem. Biophys. Res. commun.*, 294,1138-1143.

Zhen Fang, J. Z. (2013). Biodegradation of wool waste and keratinase production in scale up fermenter with different stratagies by Stenotrophomonas maltophilia BBE11-1. *Bioresource Technology*, 286-291.