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Analysis of protease activity of enzyme isolated from compost soil

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Abstract
Bacteria are very good source of enzymes as compared to animal or plant source and even synthetic enzymes. In the present investigation the protease activity has been analyzed. The source of enzyme i.e. protease producers were isolated from compost soil sample viz. collected from the Wanker farm field, Solapur. The protease producers were isolated, screened and grown on a suitable growth medium to obtain maximum production of enzyme. After production the crude enzyme is purified. The purified enzyme is analysed for its keratinolytic activity by using feathers.

Keywords: Keratin, Proteases, keratinases, MGYP broth, keratinolytic activity

INTRODUCTION

Enzyme is a biocatalyst which accelerates the rate of biological reactions. However, the concept of biocatalysts is very wide. It includes the pure enzymes, crude cell extract, and viable plant cells. There are two types of enzymes: the extracellular and intracellular enzymes. The two enzymes i.e. Protease secreted out of the cell and Keratinase remain within the cell. There is wide range of extracellular and intracellular enzymes which are of high economic value. Microbial enzymes have advantages over the animal and plant enzymes. Firstly, they are economical and can be produced on large scale within limited space and time.

At present, more than 2000 enzymes have been isolated and characterized, out of which above 1000 enzymes are recommended for various applications [1]. Among these about 50 microbial enzymes have industrial applications. Recently an application of enzymes in industries has much significance.

Proteases

Protease is an enzyme which brings about proteolysis which begins with protein catabolism by hydrolysis of the peptide chain. These enzymes are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (examples: blood clotting cascades) [2].

Keratinase [EC 3.4.21/24/99.11]

These are the enzymes which can degrade all types of keratins. Keratinases are large serine- or metallo-proteases i.e. capable of degrading the structure forming keratinous proteins. Since most of the purified keratinases known to date cannot completely solubilize native keratin [3]. Although keratinases from dermatophytic fungi have long been well known due to their notorious pathogenic nature. These enzymes have only gained biotechnological impetus recently.

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MATERIALS AND METHODS

Sample Collection

Compost soil sample was collected from the Wanker farm field, Solapur. The soil sample was collected in a sterile Petri plate. The collected sample was immediately transported to the laboratory and stored at 4^0C in refrigerator for further studies [2].

Isolation of protease producers

1gm of collected soil sample was serially diluted to obtain 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} fold dilution and 0.1ml of each dilution was inoculated by spreading on separate sterilized skimmed milk agar plates at pH 7.5 and incubated at 55^0 C for 48 hrs.

After incubation, the colonies were observed for the presence of clear zone of casein hydrolysis [4]. These colonies were subjected for purification on the same media.

Primary screening of protease producer

Primary screening of thermophilic alkaline protease producers was carried out by performing protease enzyme assay by Casein Digestion Method [5].

Production of Enzyme

For the maximum enzyme production, the isolates were selected after screening and inoculated in the MGYP broth [6].

Composition

Meat Extract	:0.5g
Glucose	:1.0g
Yeast Extract	:0.5g
Peptone	:1.0g

K_2HPO_4 :0.002g
NaCl :0.001g
Distilled Water :100.0ml
pH- :7.5

The pH of medium was adjusted to 7.5. Each isolated culture was inoculated & incubated on a water bath rotary shaker at 37˚c for enzyme production [7]. After 24 hrs, 48 hrs and 72hrs of incubation, the enzyme assay was carried out. The crude extract was carefully removed from the culture & centrifuged at 8000 rpm for 5 min. to settle down cell debris. The supernatant was carefully removed & cell pellet was discarded [7]. The supernatant i.e. crude enzyme was used for protease enzyme assay by Casein digestion method.

Fig 1. Protease enzyme production on water bath shaker viz. adjusted to 37^0 C

Protease Enzyme Assay

Protease enzyme activity was assayed by Casein Digestion Method with slight modification [8] . The assay was carried out in reaction mixture containing 1ml casein in 50mM Tris-Hcl buffer (pH 10.5) and 1ml of cell free broth solution(Crude enzyme). For blank 1 ml of distilled water was used instead of crude enzyme. The mixture was incubated at 55^0C for 30 min to carry out the reaction. After incubation the reaction was stopped by 2ml of 5% Tri-chloroacetic Acid [TCA]. Then 0.6ml of Folin-Ciocalteu's reagent was added & kept for 10min at room temperature. After incubation the mixture was centrifuged at 8000 rpm for 10 min. At last, optical density was measured at 470 nm by colorimeter and results were recorded. Protease enzyme units were calculated.

Table 1. Protease Enzyme Activity

Incubation period (days)	1	2	3	4	5	6
Optical Density (470nm)	0.23	0.53	1.53	1.63	1.62	1.12

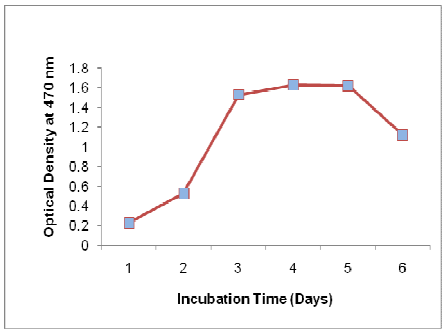

Fig 2. Determination of Casinase activity

Production of Seed culture For Kreatinolytic protease Enrichment on nutrient Broth containing Feather

For this purpose Feather Mill is subjected to nutrient Broth and the single isolated colony from casein Agar plate was Inoculated and kept on orbital shaker at 37^0 C [9].

Production of Enzyme

For maximum production the of enzyme 5ml suspension from Nutrient broth was transferred to 250 ml flask containing 100 ml feather mill medium. This flask was then incubated on orbital Rotary shaker at 37^0C at 100 rpm for respective days. Samples were withdrawn at regular intervals and centrifuged at 1000 rpm for10 min [10].The cell free extract was then stored at 4^0C and used for enzyme assay & protein estimation

Mineral Base Medium for Keratinase Production

$Mgcl_2$: 0.2g
K_2HPO_4: 0.3 g
KH_2PO_4 :0.4g
NaCl :0.5 g
Feather : 0.6g
Distilled Water :100.0ml
PH: 7.5

Protein Detection by Biuret Method

This method was used for the detection of protein within cell free extract with the help of Biuret reagent [11]. It reacts with protein Molecule, when incubated in boiling water bath for 10 min. The color changes from Blue to Violet. Soluble protein content in the culture supernatant was detected by using Biuret method and bovine serum albumin (BSA) as standard.

Determination of keratinolytic protease activity

Keratinolytic protease activity was measured by modified azocasein digest method [12]. The enzyme (4 ml) was incubated with 2 ml of 1% azocasein in 50 mMTris-HCl buffer (pH 7.5) at 37°C for 30 min in a shaking water bath. The reaction was terminated by adding 1.4 ml of 10% trichloroacetic acid (TCA) and the mixture was kept at 4°C for 15 min. After centrifugation at 10,000 rpm for 10 min the absorbance of the reaction mixture was read at 670 nm against a control prepared in the same way, except that the TCA was added before addition of enzyme. One unit of enzyme activity was defined as an increase of 0.01 absorbance units per min under the given conditions.

RESULTS

From a collected compost sample, a different bacterial isolates were obtained. The observations showed that the isolates were with prominent zone of casein hydrolysis on skimmed milk agar plate and feather degradation in broth tubes [6]

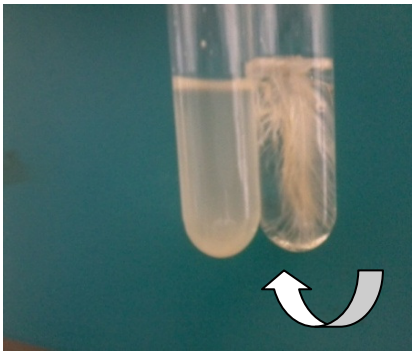

Fig 3. Determination of Keratinolytic Activity

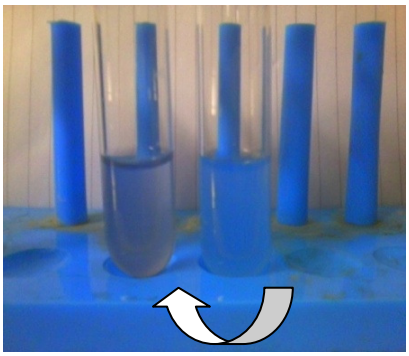

Fig 4. Biuret Activity of Purified sample

Table 2. Estimation of protein by Folin Lowery Method

Sl.no.	Stock Solution	Distilled Water	Biuret Solution	Optical Density
1	0	1	3	0
2	0.1	0.9	3	0.07
3	0.2	0.8	3	0.1
4	0.3	0.7	3	0.19
5	0.4	0.6	3	0.2
6	0.5	0.5	3	0.29
7	0.6	0.4	3	0.35
8	0.7	0.3	3	0.42
9	0.8	0.2	3	0.47
10	0.9	0.1	3	0.49
11	1	0	3	0.51
12	Sample	0.5	3	0.42

Table 3. Estimation of protein by Folin Lowery Method.

Incubation Time(Days)	Optical Density at 670nm
2	0.81
4	0.84
6	0.89
8	1.09
10	1.13
12	1.23
14	1.23
16	1.53
18	1.58
20	1.50

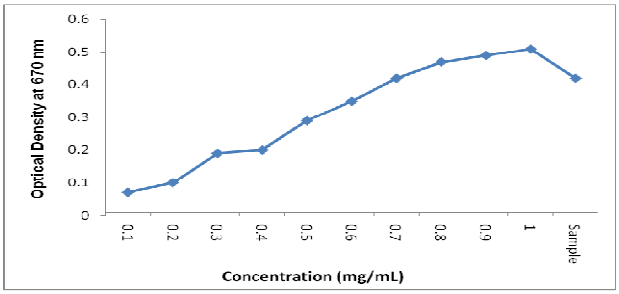

Fig 5. Estimation of protein by Folin Lowery Method

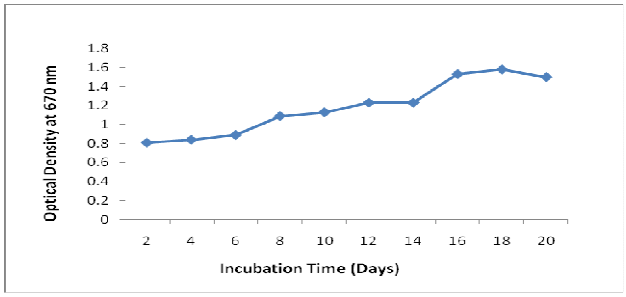

Fig 6. Estimation of protein by Folin Lowery Method

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