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 W tanker 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.

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°C 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°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
<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat Extract</td>
<td>0.5g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>0.5g</td>
</tr>
<tr>
<td>Peptone</td>
<td>1.0g</td>
</tr>
</tbody>
</table>

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Tel: +91-9766530547
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K₂HPO₄ : 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 incubated & 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°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°C 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>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Density (470nm)</td>
<td>0.23</td>
<td>0.53</td>
<td>1.53</td>
<td>1.63</td>
<td>1.62</td>
<td>1.12</td>
</tr>
</tbody>
</table>

**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]
Table 2. Estimation of protein by Folin Lowery Method

<table>
<thead>
<tr>
<th>Incubation Time (Days)</th>
<th>Optical Density at 670nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.81</td>
</tr>
<tr>
<td>4</td>
<td>0.84</td>
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<td>6</td>
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<td>10</td>
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<td>12</td>
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<td>14</td>
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<td>16</td>
<td>1.53</td>
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<tr>
<td>18</td>
<td>1.58</td>
</tr>
<tr>
<td>20</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Table 3. Estimation of protein by Folin Lowery Method

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Distilled Water</th>
<th>Biuret Solution</th>
<th>Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>0.5</td>
<td>3</td>
<td>0.42</td>
</tr>
</tbody>
</table>

REFERENCES


