Studies on Compatibility of Fungal Aalkaline Protease with Commercially Available Detergents

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Abstract: In the present endeavour, a total of 27 mold isolates were obtained from 10 soil samples by serial dilution agar plate technique and all these fungal isolates were screened for production of alkaline protease on casein agar plates. The most promising isolate was identified on the basis of cultural and microscopically characteristics as Aspergillus niger. Various cultural parameters such as substrate concentrations, enzyme concentrations, pH and temperature were observed for the optimization of production of alkaline protease. Optimum enzyme activity was observed at 1% substrate concentration, 10% enzyme concentration, at temperature 50 °C and at pH 8.5. In presence of casein, alkaline protease of Aspergillus niger retained 66-86% activity in the presence of commercially available detergents such as Tide, Surf excel, Wheel, Rin and Nirma, indicating its suitability for application in detergent industry. The lab-scale study on protease production by Aspergillus niger might give the basic information of further development for large scale production.

Keywords: alkaline protease; Aspergillus niger; detergent; optimization.

1. Introduction
Enzymes are the catalysts that perform a wide range of chemical reactions occurred in living cells, so called biocatalyst (Raimi et al., 2011). The existence of enzymes has been known for well over a century. James B. Sumner of Cornell University was able to isolate and crystallize the first enzyme urease from the jack bean (Yandri et al., 2010).

Enzymes are categorized according to the compounds they act upon. Some of the most common include: proteases which break down proteins, cellulases which break down cellulose, lipases which split fats (lipids) into glycerol and fatty acids, and amylases which break down starch into simple sugars (Jameel and Khan, 2011).

Proteases are enzymes that catalyze the degradation of peptides and proteins, and occupy a significant role in physiologic processes in the living beings, as well as by their use in different industrial processes. In the world, the industries that apply enzymes for their products invest annually near a trillion of dollars in their commercialization. Of them, 75% belong to hydrolytic enzymes and of this percentage, the proteases represent 60% of total of the world-wide sales (Liggieri et al., 1998). According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, proteases are classified in subgroup 4 of group 3 (hydrolases) (Rao et al., 1998).

In 1960, Dane first isolated alkaline protease from *Bacillus licheniformis*. Proteases have been isolated and purified from microorganisms, animals and plants; among them microorganisms represent the most common source of enzymes because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation (Zhang and Kim, 2010). Two third of the industrial produced proteases are from microbial sources (Adinarayana and Ellaiah, 2002). The various microorganisms such as bacteria, fungi, yeast and *Actinomycetes* are known to produce these enzymes (Madan et al., 2002; Devi et al., 2008). Molds of the genera *Aspergillus*, *Penicillium* and *Rhizopus* are especially useful for producing proteases, as several species of these genera are generally regarded as safe (Devi et al., 2008). *Aspergillus clavatus* ES1 has been recently identified as a producer of an extracellular bleaching stable alkaline protease (Hajji et al., 2008).

Proteases are extensively used in numerous fields including protein engineering (Durrschmidt, 2001), food processing industry, leather industry, silk industry, detergent industry (Joo, 2005) and waste processing industry (Pastor, 2001). Proteases regulate the fate, localization, and activity of many proteins, modulate protein-protein interactions, create new bioactive molecules, contribute to the processing of cellular information. Accordingly, many proteases are a major focus of attention for the pharmaceutical industry as potential drug targets or as diagnostic and prognostic biomarkers (Otín and Bond, 1999).

As detergent additives, alkaline proteases dominate commercial applications market. Alkaline proteases added to laundry detergents enable the release of proteinaceous material from stains. The
increased usage of these proteases as detergent additives is mainly due to the cleaning capabilities of these enzymes was environmentally acceptable (Kumar and Takagi, 1999).

In lieu of above justification, the objectives of the present study were as follows: (1) isolation and purification of fungi from soil samples, (2) screening of soil fungi for the production of alkaline protease, (3) optimization of the production of alkaline protease from the most promising fungus, and (4) determination of compatibility of fungal protease with commercially available detergents.

2. Materials and Methods

2.1. Isolation of Molds from Soil

2.1.1. Collection of soil samples

A total of 10 soil samples were collected from crop fields, garden and domestic wastes from Ambala, Haryana, India. Soil samples were collected in sterile polyethylene bags, labelled with date and site of collection, brought to the laboratory and stored in the refrigerator.

2.1.2. Serial dilution agar plate technique for isolation of molds

Isolation of molds from the soil was done by a modified serial dilution agar plate technique. Potato dextrose agar (PDA) supplemented with streptopenicillin as antibacterial agent was used for the isolation of soil molds. Ten grams of soil (finely pulverized and air dried) was suspended in 90 mL sterilized distilled water (blank no. 1) and shaken vigorously on a magnetic stirrer for 20 min to obtain uniform suspension of soil molds. The 10 mL of suspension was transferred, while in motion, from the stock suspension (no. 1), into sterile water blank number 2 with sterile pipette under aseptic conditions to make 1:100 (10^-2) dilution and shaken well for about five minutes. Further dilutions from (10^-3) to (10^-7) were made by pipetting 10 mL suspension into sterile water blanks number 3, 4, 5, 6 and 7 from water blank number 2, 3, 4, 5 and 6, respectively. Finally 1 mL aliquots of the suspension of final five dilutions i.e. (10^-3) to (10^-7) were added to labelled and sterilized Petri plates. Approximately, 25 mL cooled molten (45°C to 50°C) PDA supplemented with streptopenicillin, was added to each Petri plate and mixed gently by rotation. After solidification of the medium, the inoculated Petri plates were incubated in an inverted position at 25°C for 3 to 7 days and observed for the appearance of mold colonies produced on each plate of different dilutions. The purification of soil molds was done by needle inoculation and disc transfer methods on PDA plates. The inoculated PDA plates were incubated at 25 °C for 5 days and observed for purity and pure cultures were subcultured on PDA slants. The inoculated slants were observed for the growth of pure cultures and maintained at 4 °C in a refrigerator (Pundir and Jain, 2010).
2.2. Screening of Isolates for Alkaline Protease Production

Screening of protease production was done on casein plate assay as per (Upadhyay et al., 2002). Each isolate was inoculated in Petri plate containing PDA with 1% casein and inoculated at 28 °C for 5 days. The plate was flooded with 15% of HgCl₂ and result was observed (Upadhyay et al., 2010). The alkaline protease production was confirmed by formation of clear zones of hydrolysis around the colonies. The organism with maximum zone formation was further analyzed (Kumar et al., 2012).

2.3. Identification of Molds

Mold isolates were identified by cultural and microscopically characterizations and using Lactophenol cotton blue mounting. Result was observed under microscope (Muthulakshi et al., 2011).

2.4. Production of Alkaline Protease from Most Promising Fungus

Fermentation conditions: A. niger culture was maintained on potato dextrose media at 4 °C. Fungal spores were obtained from 5-7 day old culture grown on PDA at 30 °C. The spores were collected in 0.01% (w/v) Tween-80 solution. The suspension of 10³ spores were inoculated into the 250 mL Mandels and Reese Broth at a pH of 8-8.5, and then incubated on a rotary shaker at 30 °C, 250 rev/min for 10 days. The mycelia were separated from the fermentation broth by centrifugation at 6,000 rpm for 15 min and the clear supernatant was used as the crude enzyme extract (Muthulakshmi et al., 2011).

2.5. Protein estimation by Lowry’s Method

Protein was estimated by method as described by Lowry et al. (1951).

2.6. Enzyme Assay and Optimization of Alkaline Protease from A. niger

2.6.1. Effect of substrate concentrations on enzyme activity

The reaction mixtures containing various concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5%) of 0.5 mL casein and 3 mL of 0.2 M Tris HCl buffer, pH 8.5 and 1.0 mL enzyme were incubated at 50 °C. After 10 min, the reaction was stopped by adding 3 mL of cold 10% trichloroacetic acid. After 1 h, the culture filtrate was centrifuged at 8000 rpm for 5 min to remove the precipitate. Then 5 mL of reagent C was added and the set up was incubated at 37 °C for 10 min. The 0.5 mL of FC reagent was added and kept for 30 min for 37 °C. Absorbance was read spectrophotometrically at 630 nm. Enzyme activity was calculated by measuring mg of tyrosine equivalent released and compared with the
standard. One unit (U) of enzyme activity represents the amount of enzyme required to liberate 1 μg of tyrosine under standard assay condition (Upadhyay et al., 2010).

2.6.2. Effect of Enzyme Concentrations on Enzyme Activity

Similarly another set of reaction mixture was prepared containing a constant volume of 0.5 mL of 1% casein and 3 mL of 0.2 (M) Tris HCl buffer, pH 8.5 and different concentrations of enzyme (5%-25%) was incubated at 50 °C. After 10 min, the reaction was stopped by adding 3 mL of cold 10% TCA. After 1 h, the culture filtrate was centrifuged at 8000 rpm for 5 min to remove the precipitate and then 5 mL of reagent C was added and the set up was incubated at 37 °C for 10 min. The 0.5 mL of FC reagent was added and kept at 30 °C for 30 min. Absorbance was read spectrophotometrically at 630 nm (Jameel et al., 2011).

2.6.3. Effect of pH on the enzyme activity

The above reaction mixture was prepared containing a constant volume of 0.5 mL of 1% casein and 3 mL of 0.2 (M) Tris HCl buffer, of varying pH (from pH 5 to pH 11) and 1.0 mL of enzyme was incubated at 50 °C. After 10 min, the reaction was stopped by adding 3 mL of cold 10% TCA. After 1 h, the culture filtrate was centrifuged at 8000 rpm for 5 min to remove the precipitate and then 5 mL of reagent C was added and the set up was incubated at 37 °C for 10 min. The 0.5 mL of FC reagent was added and kept at 30 °C for 30 min. Absorbance was read spectrophotometrically at 630 nm (chanda et al., 2011).

2.6.4. Effect of temperatures on the activity of enzyme

The 0.5 mL of 1% casein and 3 mL of 0.2 M Tris HCl buffer (pH 8.5) and 1.0 mL of enzyme was treated at different temperature ranging from 20 °C to 70 °C. After 10 min, the reaction was stopped by adding 3 mL of cold 10% TCA. After 1 h, the culture filtrate was centrifuged at 8000 rpm for 5 min to remove the precipitate and then 5 mL of reagent C was added and the set up was incubated at 37 °C for 10 min. The 0.5 mL of FC reagent was added and kept for 30 min for 37 °C. Absorbance was read spectrophotometrically at 630 nm (Chanda et al., 2011).

2.7. Study of Compatibility with Commercially Available Detergents

Detergent solutions (Tide, Surf excel, Fena, Nirma, Rin) at a concentration of 0.07 mg/mL were prepared in distilled water. These solutions were boiled for 10 min to destroy any protease already present and cooled. A reaction mixture was prepared which contained 0.5 mL of 1.5% casein as a substrate; 3 mL of 0.2 M Tris HCl buffer (pH 8.5) and 0.5 mL of detergent solution (0.07 mg/mL)
were added. After 10 min incubation, 1.0 mL of enzyme solution was added and kept at 50 °C for 1 h. Then 3 mL of 10% of TCA solution was added in each test tubes, kept at 50 °C for 30 min. The culture filtrate was centrifuged at 8000 rpm for 5 min to remove the precipitate and then 5 mL of reagent C was added and the set up was incubated at 37 °C for 10 min. The 0.5 mL of FC reagent was added and kept for 30 min at 37 °C. Absorbance was read spectrophotometrically at 630 nm (Adinarayana et al., 2003).

3. Results

3.1. Screenings of Fungal Isolate for Protease Production

A total of 27 mold isolates were obtained from 10 soil samples by serial dilution agar plate technique. Pure isolates were maintained at 4 °C for further studies. All the 27 fungal isolates were screened for the production of alkaline protease (Fig. 1). Of the 27 isolates, 7 isolates (isolate no. 4, 7, 10, 14, 16, 21 and 25) did not show any zone of hydrolysis while 10 isolates (isolate no. 11, 3, 5, 11, 12, 17, 18, 23, 26 and 27) showed minor zone of hydrolysis, 8 isolates (isolate no. 2, 6, 8, 9, 13, 19, 22 and 24) showed moderate zone of hydrolysis and 2 isolates (isolate no. 15 and 20) exhibited good zone of hydrolysis (Table 1). Out of isolate no. 15 and 20, isolate no. 20 was found to be most effective as shown by good zone of hydrolysis.

<table>
<thead>
<tr>
<th>S. No.</th>
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<th>Observation</th>
<th>S. No.</th>
<th>Isolate No.</th>
<th>Observation</th>
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<tr>
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<td>-</td>
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<td>Isolate No. 3</td>
<td>+</td>
<td>17</td>
<td>Isolate No 17</td>
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<td>Isolate No. 4</td>
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<td>18</td>
<td>Isolate No 18</td>
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<td>+</td>
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<td>++</td>
<td>27</td>
<td>Isolate No27</td>
<td>+</td>
</tr>
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</table>
| 14     | Isolate No. 14 | -          |  |}

Note: - = No zone of hydrolysis; + = Minor zone of hydrolysis; ++ = Moderate zone of hydrolysis; +++ = Good zone of hydrolysis.
3.2. Identification of Most Promising (Alkaline Protease Producing) Fungus

On the basis of cultural and microscopical characteristics the fungal isolate no. 20 was identified as *Aspergillus niger*. The colonies colour was dark black on PDA medium and microscopically conidiophore arises from foot cells. Conidiophores bearing vesicles (globose vesicle) were dark black in colour due to black conidia (single or in chain) having uniseriate phialides (Figs. 2 & 3).

![Figure 1](image1.png)

**Figure 1.** Casein hydrolysis assay: (A) Control (casein agar without fungus), (B) Clear zone of hydrolysis produced around the fungal colonies.

![Figure 2](image2.png)

**Figure 2.** *Aspergillus niger* purified by streak plate method

![Figure 3](image3.png)

**Figure 3.** Pure culture of *Aspergillus niger* on PDA medium

3.3. Production of Alkaline Protease from A. niger

The mycelia were separated from the fermentation broth by centrifugation at 6,000 rpm for 15 min and the supernatant produced after centrifugation was used as the crude enzyme (Figs. 4 & 5).
Figure 4. Showing the mycelial growth after incubation of 10 days

Figure 5. Crude enzyme in supernatant after centrifugation

3.4. Protein Content Determination (Lowry’s Method)

The protein content of *A. niger* alkaline protease in a solution was determined to be 0.53 μg/mL obtained from BSA standard curve shown in Graph 1.

Graph 1. Lowry’s method graph showing the concentration of protein in the sample.

3.5. Optimization of the Production of Alkaline Protease

3.5.1. Enzyme assay

One unit (U) of enzyme activity represents the amount of enzyme required to liberate 1 μg of tyrosine under standard assay condition. According to the standard curve of tyrosine (Graph 2), the activity was 35 U/mL.
3.5.2. Effect of substrate concentrations on protease activity

The various concentrations of substrate (casein) ranging from 0.5 to 3.5 % with respect to its optimum time were observed. The Graph 3 showed the rate of reaction declined if the substrate concentration was more than 1% for A. niger protease. The declination in the rate of reaction after optimum concentration could be due to the alteration of enzyme substrate concentration ratio.

3.5.3. Effect of Enzyme concentrations on protease activity

Graph 4 shows the effect of various enzyme concentrations (5-25 %) on protease activity of A. niger observed that the activity increased with the increase of enzyme concentration up to 10 % and then decreased. The maximum reaction velocity at 10 % enzyme concentration is due to the utilization of total amount of substrate in enzyme-substrate complex.
3.5.4. Effect of temperatures on protease activity

The effect of temperatures on protease enzyme activity was observed and the results are shown in Graph 5. The temperature profile indicates that the optimum temperature of reaction for *A. niger* protease was found to be 50 °C. An initial increase in temperature increased the rate of the enzyme catalyzed reaction due to the increase in number of collision between the reacting molecules, but the enzyme probably got denatured on exposure to higher temperature than its optimum temperature.

3.5.5. Effect of different pH on protease activity

The effect of pH on the activity of alkaline protease was studied with various pH values ranging from 8-11 (Graph 6). The optimum pH for alkaline protease enzyme from *Aspergillus niger* was determined as 8.5. This proves the alkaline nature of the enzyme.

![Graph 5. Effect of temperature on enzyme activity.](image1)

![Graph 6. Effect of pH on enzyme activity.](image2)

3.6. Compatibility of Alkaline Protease with Commercial Detergents

Compatibility of alkaline protease was studied with various commercial detergents in presence of substrate. Alkaline protease of *A. niger* retained 66.66% to 86.66% activity in presence of different detergents, respectively (Table 2 and Graph 7).

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Protease source</th>
<th>OD at 630</th>
<th>Control OD</th>
<th>% Activity Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><em>A. niger</em></td>
<td>0.015</td>
<td>0.015</td>
<td>100</td>
</tr>
<tr>
<td>Rin</td>
<td><em>A. niger</em></td>
<td>0.011</td>
<td>0.015</td>
<td>73.33</td>
</tr>
<tr>
<td>Nirma</td>
<td><em>A. niger</em></td>
<td>0.012</td>
<td>0.015</td>
<td>80</td>
</tr>
<tr>
<td>Tide</td>
<td><em>A. niger</em></td>
<td>0.010</td>
<td>0.015</td>
<td>66.67</td>
</tr>
<tr>
<td>Surf excel</td>
<td><em>A. niger</em></td>
<td>0.012</td>
<td>0.015</td>
<td>80</td>
</tr>
<tr>
<td>Fena</td>
<td><em>A. niger</em></td>
<td>0.013</td>
<td>0.015</td>
<td>86.67</td>
</tr>
</tbody>
</table>

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The results indicated that the fungal protease was found to be compatible with detergents. The fungal protease showed maximum activity with Fena followed by Nirma, Surf excel, Rin and minimum with Tide (Graph 7).

Graph 7. Effects of different detergents on enzyme activity.

4. Discussion

Enzyme production by microorganisms is greatly influenced by media components, especially carbon and nitrogen sources, and physical factors such as temperature, pH, and incubation time and inoculums density. It is important to produce the enzyme in inexpensive and optimized medium on a large scale for the process to be commercially viable; hence the studies on the influence of various physicochemical parameters such as incubation periods, inoculum size, temperature, pH, and carbon source were carried out (Muthulakshmi et al., 2011). Microbial proteases have a number of commercial applications in industries like food, leather, meat processing and cheese making. A major commercial use is the addition of microbial proteases to domestic detergents for the digestion of pertinacious stains of fabrics (Sharma et al., 1980). It has been reported that the production of extracellular proteases by different microorganisms can be strongly influenced by the culture conditions. So, it becomes necessary to understand the nature of proteases and their catalytic potentiality under different conditions (Muthulakshmi et al., 2011).

A total of 27 molds isolate were obtained from 10 soil sample by serial dilution agar plate technique. These 27 molds were screened for production of alkaline protease by casein plate method. Isolate no. 20 was found to be most effective as shown by good zone of hydrolysis. Colony morphology, microscopic observation and culture characteristics have confirmed the identity of the
fungi as *Aspergillus niger*. Further alkaline protease was isolated from *A. niger* by different methods and cultural parameters are optimized one by one taking other parameters constant.

The protein content of *A. niger* alkaline protease in a solution was determined to be 0.53 microgram per mL. The protein content was determined by BSA standard curve. Results revealed that alkaline protease from *Aspergillus niger* had a optimum substrate concentration 1%. Negi and Banerjee (2010) have reported optimum substrate concentration for proteases production with casein as a substrate for *A. niger* was 1%.

In the present study, the effect of various enzyme concentrations on protease activity of *A. niger* was observed that the activity increased with the increase of enzyme concentration up to 13-15% and then decreased. The maximum reaction velocity at 15% enzyme concentration is due to the utilization of total amount of substrate in enzyme-substrate complex (Sandra and Pant, 2005). Our results are accordance with Negi and Banerjee (2010) who have reported enzyme concentration for proteases from *A. niger* was 14%.

During the study protease production increased with increase in temperature from 45 to 50 °C. Maximum production of protease was obtained at 50 °C. Growth and protease production ceased at higher temperature (60 °C), and similar observation was shown by Morimura *et al.* (1994) for *Aspergillus usami*. It was revealed that environmental temperature not only affects growth rates of organism but also exhibit marked influence on the levels of protease production. In addition, the enzyme was completely inactivated at 70 °C. Li *et al.*, (1997) reported that alkaline protease isolated from *Thermomyces lanuginose* P134 had a broad temperature optimum of 50 °C. Thammawa *et al.* (2007) reported the optimum temperature for protease from *A. niger* was in the range between 45 and 50 °C.

Protease produced by *Aspergillus niger* was observed with respect to the range of pH between 8 and 9. Maximum protease production was found at pH 8.5. The results clearly indicated alkalinophilic nature of the fungus and the protease. Optimum pH 8.4 has been reported for alkaline protease of *Conidiobolus coranaulis*. Likewise pH 7 has been reported to be optimum for *Aspergillus flavus* (Sutar *et al.*, 1992). The effect of pH on the activity of alkaline protease was studied with various pH from 8-11. The optimum pH for alkaline protease enzyme from *Aspergillus niger* was determined as 9 (Negi and Banerjee, 2010). These findings are in accordance with earlier reports showing pH optima of 10.0 to 10.5 for protease from *Bacillus* species, *Thermus aquaticus*, *Xanthomonas maltophilia* and *Vibrio mettsonikovii* (Durham *et al.*, 1987).

One unit (U) of enzyme activity represents the amount of enzyme required to liberate 1 μg of tyrosine under standard assay condition. The enzyme activity determined as per tyrosine standard was
35 U/mL for \textit{A. niger}. In case of \textit{A. niger} the activity was to be reported 34 U/mL by (Madan \textit{et al.}, 2002) and 42 U/mL in case of \textit{Aspergillus flavus} (Sutar \textit{et al.}, 1992).

Enzyme activity and stability in presence of some available commercial detergents was studied with a view to exploit the enzyme in detergent industry. As shown in (Table 2 and Graph 7), in presence of substrate, alkaline protease of \textit{A. niger} retained 66.66\% to 86.66\% activity in presence of different detergents. The highest activity was retained in presence of Fena and the least activity was observed in case of Tide.

Madan \textit{et al.} (2002) studied the compatibility of alkaline protease from \textit{Bacillus polymyxa} retained 20-84.5\% of its activity in various detergents. Adinarayana (2003) also reported 16, 11.4 and 6.6\% activity in Revel, Ariel and Wheel respectively. In an another study, enzyme was found to be 100\% stable in presence of commercially available detergents like Surf and Henko and 85\% stable in presence of Ariel and Tide. Stability of protease for laundry detergents up to 80\% was earlier reported by Venugopal and Saramma (2006) for alkaline protease produced by \textit{Vibrio fluvialis} strain VM10 isolated from mangrove sediment sample (Sindhu \textit{et al.}, 2009).

Based on the results reported here, it is proposed that the alkaline protease produced by \textit{Aspergillus niger} isolated by us may have great commercial value as sufficiently high levels of enzyme activity and high stability in the presence of detergents. The appreciably high enzyme activity makes this isolate an industrially promising and of special interests for basic and applied research.

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\section{References}


Upadhyay, M. K., Kumar, R., Kumar, A., Gupta, S., Kumari, M., Singh, A., Jain, D., and Verma, H.
