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# ISOLATION, OPTIMIZATION AND PRODUCTION OF PROTEASE FROM *ASPERGILLUS* SPECIES THROUGH SOLID STATE FERMENTATION

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## ABSTRACT

The production of enzymes by bioprocesses is a good value added to agro industry residues. A comprehensive study was carried out on the production of protease using different agricultural wastes like paddy straw, sugarcane bagasse, peanut hull and rice bran by *Aspergillus* species. Among the all tested the maximum enzyme production was observed in paddy straw, while minimum protease production noticed in rice bran under solid state fermentation conditions. The optimal conditions for producing maximum yield of protease were incubated at $35^0$C, 4 days, pH 6.The protease production from waste treatment could be commercially used in detergents and leather industry.

## INTRODUCTION

Enzymes are delicate protein molecules necessary for life. Protease is the single class of enzymes which occupy pivotal position due to their wide applications in detergents, pharmaceuticals, photography, leather, food and agricultural industries and representing worldwide sale at about 60% of total enzyme market (Paranthaman *et al*., 2009; Rajmalwar and Dabholkar, 2009; Das and Prasad, 2010). Proteases of fungal origin have an advantage over bacterial protease as mycelium can be easily removed by filtration. Proteases produced by *Aspergillus* sp. is of greater importance due to its higher protease producing ability (Chakraborty *et al*., 1995; Nehra *et al.*, 2002). Solid state fermentation (SSF) has many advantages including superior volumetric downstream processing, lower energy requirement and low wastewater output (Malathi and Chakraborty, 1990; Pandy *et al*., 1999). The present study was undertaken to produce protease under laboratory conditions by solid state fermentation of *Aspergillus* sp. using paddy straw, sugarcane bagasse, peanut hull and rice bran as substrate and to determine the effect of pH, temperature and incubation period on protease production.

## MATERIALS AND METHODS

### Isolation of *Aspergillus* sp:

For isolation of *Aspergillus*, rhizosphere soil samples were collected from paddy fields of Cherlopalli, near Tirupati area of Andhra Pradesh. The collected samples were subjected to serial dilution method by using potato dextrose agar medium. Then the isolate was screened for their proteolytic activity by using Skimmed Milk Agar (SMA) medium and maintained on PDA slants for further use.

**Production of protease though solid state fermentation:**

**Inoculum preparation:**

Three ml of 0.1% Tween 80 was added to release the spores and this spore suspension was used as inoculums for fermentation.

**Substrate preparation and inoculation:**

Four substrates i.e., paddy straw, sugarcane bagasse, peanut hull and rice bran were used for protease production. 5 g of each substrate was taken into two separate was taken in separate 250 ml conical flasks and salt solution was added to maintain 70% moisture. Then the flasks were sterilized at $121^0$C for 15 min. The above flasks were inoculated with 1 ml of inoculum and incubated at room temperature for 5 days.

**Extraction of crude enzyme:**

Seventy five ml of double distilled water was added to the conical flasks and kept on rotary shaker for about half hour to obtain uniform suspension. The suspension was filtered through Whatman No: 1 filter paper and the filtrate were collected separately and used as an enzyme extract.

**Assay for neutral protease:**

To 200 µl of crude enzyme extract, 500 µl of 1% casein and 300 µl of 0.2 mol/l phosphate buffer (pH 7.0) were added. The reaction mixture was incubated at $60^0$C for 10 min and arrested by the addition of 1 ml of 10 % Trichloroacetic acid (TCA). The reaction mixture was centrifuged at 8000 x g for 15 min and to the supernatant, 5 ml of 0.4 ml $Na_2CO_3$, 1 ml of 3 fold diluted Folin Ciocalteau's phenol reagent was added. The resulting solution was incubated at room temperature for 30 min and the absorbance of the blue colour developed was read at 660 nm using a tyrosine standard. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µg of tyrosine from substrate (casein) per minute under assay conditions and reported in terms of protease activity per gram dry fermented substrate.

**Effect of pH:**

Different levels of pH i.e., 4.0, 5.0, 6.0 and 7.0 were evaluated for protease production of four substrates by using *Aspergillus* sp.

**Effect of temperature:**

The inoculated substrates were incubated at different temperatures viz., 20, 30, 40, and 50 to find the effect of temperature on protease production.

**Effect of Incubation period:**

The effect of incubation period on protease production was determined by incubating the production medium for different incubation periods viz., 3, 4, 5 and 6 days, respectively.

## RESULTS AND DISCUSSION

The process parameters for the production of protease by *Aspergillus s*p. grown on different substrates (paddy straw, sugarcane bagasse, peanut hull and rice bran) was done under optimized condition (Sudto *et al*., 2008; Gitishree Das and Prasad., 2010; Vishalakshi *et al*., 2009). In the present study the maximum enzyme production was observed in paddy straw, while minimum protease production noticed in rice bran. As shown in Table 1, pH showed effect on protease production because microbial strains depends on extracellular pH which strongly influences the many enzymatic processes and transport of various components across the cell membrane which in turn support the cell growth and product production (Paranthaman *et al*., 2009).

**Table 1: Effect of different ph of different on protease production by *Aspergillus* species**

| Substrates | Different pH values | | | |
|---|---|---|---|---|
| | 4.0 | 5.0 | 6.0 | 7.0 |
| **Paddy straw** | 1.9 | 2.4 | 4.8 | 3.4 |
| **Sugarcane bagasse** | 1.6 | 2.1 | 4.6 | 3.0 |
| **Peanut hull** | 1.3 | 2.0 | 4.1 | 2.9 |
| **Rice bran** | 1.2 | 2.2 | 4.1 | 2.7 |

The optimum pH for growth was recorded at pH 6 in all substrates. A notable decline in the enzyme productivity occurred at both high and lower pH values. Similar results were also reported by several works (Paranthaman *et al*., 2009; Teufel and Gotz., 1993; Vishalakshi *et al*., 2009). Temperature also showed maximum variation in the protease production (Tab 2).

**Table 2: Effect of different temperatures on protease production by *Aspergillus* species**

| Substrates | Different temperatures($^0$C) | | | |
|---|---|---|---|---|
| | 20 | 30 | 40 | 50 |
| **Paddy straw** | 1.7 | 3.1 | 2.0 | 1.6 |
| **Sugarcane bagasse** | 1.4 | 2.5 | 2.2 | 1.5 |
| **Peanut hull** | 1.2 | 2.3 | 2.0 | 1.2 |
| **Rice bran** | 1.3 | 2.8 | 2.4 | 1.4 |

**Table 3: Effect of different incubation period on protease production by using *Aspergillus* species**

| Substrates | Incubation period(days) | | | |
|---|---|---|---|---|
| | 3 | 4 | 5 | 6 |
| **Paddy straw** | 1.8 | 2.2 | 3.4 | 2.5 |
| **Sugarcane bagasse** | 1.5 | 2.3 | 3.4 | 2.2 |
| **Peanut hull** | 1.2 | 2.1 | 3.0 | 2.1 |
| **Rice bran** | 2.2 | 2.5 | 3.2 | 2.0 |

The maximum activity was found at 30$^0$C in all the substrates. Results in the table 3 indicate that maximum enzyme production was observed at 5 days of incubation period in all the substrates (Rajmalwar, S. and Dabholkar, P.S., 2009). A gradual decrease in enzyme units was observed with increasing incubation period clearly suggests that enzymes role as a primary metabolite

being produced in the log phase of the growth of the fungus for utilization of proteins present in the solid substrates (Sudto *et al*., 2008; Gitishree Das and Prasad., 2010; Vishalakshi *et al*., 2009). The subsequent decrease in the enzyme production could be probably due to inactivation of the enzyme by other constituent protease.

## CONCLUSION

The pH, temperature and incubation periods showed much effect on production of protease by *Aspergillus* species.

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