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Production of Protease from Rice Mill Wastes by *Aspergillus niger* in Solid State Fermentation

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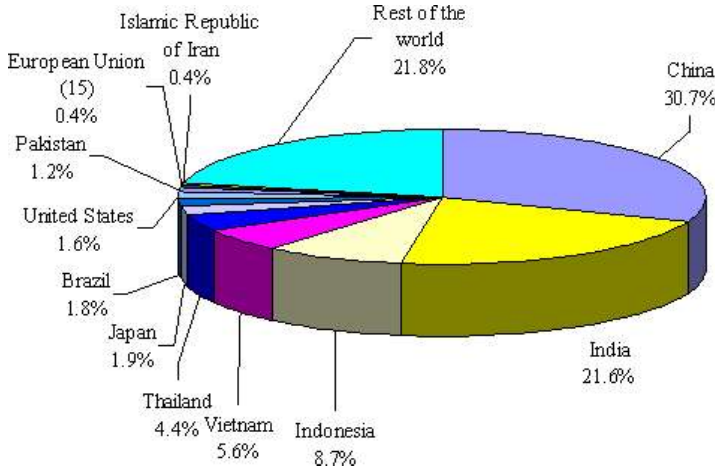
Abstract: The production of enzymes by bioprocesses is a good value added to agro industry residues. A comparative study was carried out on the production of protease using different varieties of Rice brokens (PONNI, IR-20, CR-1009, ADT-36 and ADT-66) from Rice mill wastes as substrates in solid-state fermentation (SSF) by *Aspergillus niger*. Among the all tested varieties of rice broken PONNI produced the highest activity as 67.7 U/g while ADT-66 produced lowest protease as 44.7 U/g/ under solid state fermentation conditions. The optimized conditions for producing maximum yield of protease were incubation at 35°C, 96 h and pH 7.0. The protease production from waste treatment could be commercially used in detergents and leather industry.

Key words: Solid state fermentation · Rice mill wastes · Protease

INTRODUCTION

Rice cultivation is the principal activity and source of income for millions of households around the globe. Several countries of Asia and Africa are highly dependent on rice as a source of foreign exchange earnings and government revenue. Asia is the biggest rice producer, accounting for 90% of the world's production and consumption of rice. China and India, which account for more than one-third of global population supply over half of the world's rice. (Fig.1) Broken rice is the rice kernel that does not survive the milling process. They have length dimensions smaller than ¾ of the whole grains. Rice kernels can develop cracks in the field during drying or during milling, quick drying and over drying (below moisture content levels of 12%) or dehydrating oven dried kernels are the major causes for the breakage of kernels during milling. Most broken rice was used for beer making in the past. But, nowadays, high quality of brewers prefers whole rice than broken rice for beers. Broken rice is used as feed for live stock or for making pet foods. Some lower brewers still use broken rice. This low valued by-product of rice milling industry can effectively be used for better economic returns and are, for instance, alternate uses of the rice broken kernels.



Source: UNCTAD Secretariat from the Food and Agriculture Organization of the United Nations (FAO) data

Fig. 1: Distribution of the world paddy rice production (average 1999-2003)

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Enzymes commercially available now are not economically comparable to the chemical process. Hence, any substantial reduction in the cost of production of enzymes will be a positive stimulus for the commercialization of enzymatic depilation. Proteases are one of the most important groups of industrial enzymes and account for nearly 60% of the total enzyme sale [1,2]. The major uses of free proteases occur in dry cleaning, detergents, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds [3]. Solid-substrate fermentation (SSF) was chosen for the present research because it has been reported that previously as much greater productivity than does submerged fermentation Ghildyal *et al*., [4] and Hesseltine, [5]. Economically, SSF offers many advantages, including superior volumetric productivity, use of simpler machinery, use of inexpensive substrates, simpler downstream processing and lower energy requirements [6,7] with submerged fermentation [8].

The present study was undertaken to produce the proteases under solid state fermentation of *Aspergillus niger* using rice broken as a substrate,and to determine the effect of pH, temperature and incubation time on protease production.

MATERIALS AND METHODS

Substrate: The substrate Rice broken of different varieties i.e. PONNI, IR-20, CR-1009, ADT-36 and ADT-66 were obtained during milling process of rice mills in Indian Institute of Crop Processing Technology, Thanjavur.

Microorganism and Maintenance of Culture: The organism used in the present study was *Aspergillus niger* MTCC 281 which kindly obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh, India. The culture was routinely maintained on potato dextrose agar slants. The organism was subculture for every month.

Inoculum Preparation: The inoculum was prepared by dispersing the spores from a week-old fungal slant culture in 0.1 % Tween-80 solution with a sterile inoculation loop.

Solid-State Fermentation: Five grams of each tested variety of rice broken was taken in a 250 ml Erlenmeyer flask seperately, moistened with 10 ml of the salt solution [composition (% w/v) (g/100ml): ammonium nitrate 0.5, potassium dihydrogen orthophosphate 0.2, sodium chloride 0.1 and magnesium sulphate 0.1], sterilized at 121.5°C for 15 min, cooled, inoculated with 1 ml of fungal spore suspension (10^6 spores/ml) and incubated at 35°C for 120 hr.

Extraction of Crude Enzyme: A solution of Tween-80 (0.1 %) was added in to the 100 ml of distilled water. 10 ml of the water was added to the 2 g of fermented substrate and the substrate was homogenized on a rotary shaker at 180 rpm for 1 h. The solids were removed by centrifuging the homogenate at 8000 x *g* at 4°C for 15 min and the resultant clear supernatant was used for analytical studies.

Assay for Neutral Protease: To 200 µl of crude enzyme extract, 500 µl of casein (1 %) and 300 µl of 0.2 mol/l phosphate buffer (pH 7.0) were added. The reaction mixture was incubated at 60 °C for 10 min and arrested by the addition of 1 ml of 10 % trichloroacetic acid [9]. The reaction mixture was centrifuged at 8000 x *g* for 15 min and to the supernatant, 5 ml of 0.4 mol/l Na_2CO_3,1 ml of 3-fold diluted Folin and Ciocalteau's phenol reagent was added. The resulting solution was incubated at room temperature for 30 min and the absorbance of the blue color developed was read at 660 nm using a tyrosine standard [10]. 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 Incubation Period: The effect of incubation period on protease was determined by incubating production medium for different incubation periods viz. 24, 48, 72, 96 and 120 h.

Effect of Incubation Temperature: The inoculated substrates were incubated at different temperatures viz.20, 25, 30, 35 and 40 °C to find the effect of temperature on protease production.

Effect of pH: Different levels of pH i.e 5.0, 6.0, 7.0&8.0 were evaluated for protease production.

RESULTS AND DISCUSSION

The process parameters for the production of protease by *A.niger* grown on different varieties of rice broken (PONNI, IR-20, CR-1009, ADT-36 and ADT-66) as a substrates were done under optimized conditions. The results are presented and discussed as follows.

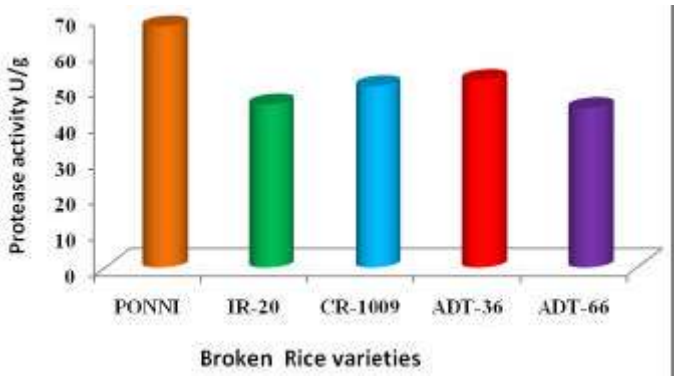

Fig. 2: Protease production from rice broken by Solid State fermentation

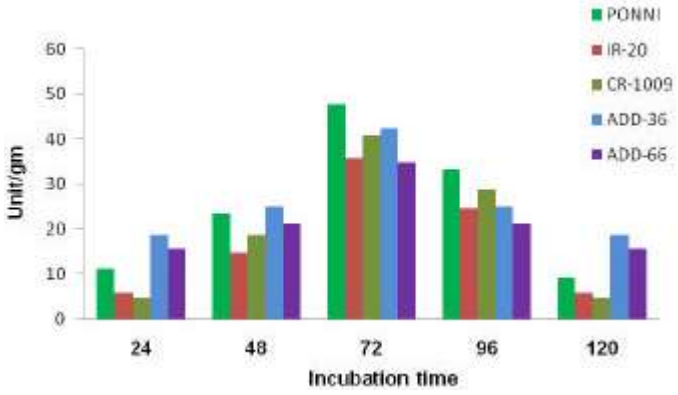

Fig. 3: Effect of incubation period on the production of protease enzyme using different varieties of rice broken used as solid substrate.

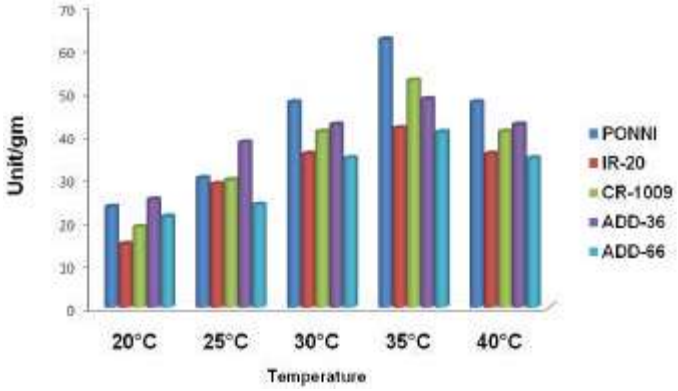

Fig. 4: Effect of different incubation temperature on the production of protease enzyme different rice broken varieties used as solid substrate

Protease Activity: The selection of an ideal agro-biotech waste for enzyme production in a solid-state fermentation process depends upon several factors, mainly related with cost and availability of the substrate material and thus may involve screening of several agro-industrial residues [11]. In the present study the maximum enzyme production of 67.7 U/g biomass was observed in PONNI, while minimum protease production 44.7 U/g biomass was noticed with ADT-66 variety (Fig. 2).

Effect of Incubation Period: Result of this study showed that protease production increased with incubation period. Maximum enzyme production was observed at 72 h of incubation (Fig. 3) in all varieties of rice broken.

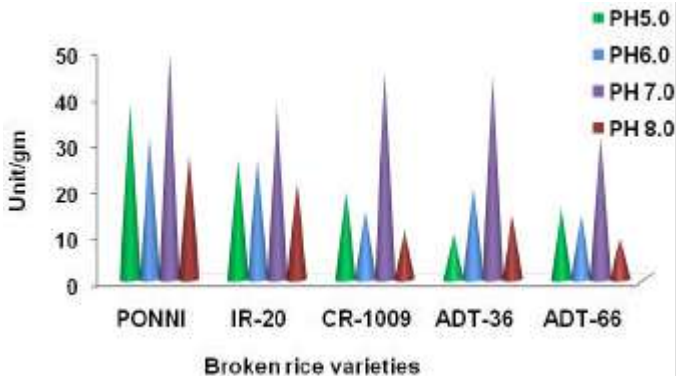

Fig. 5: Effect of pH values on the production of protease enzyme on rice broken used as solid substrate

A gradual decrease in enzyme units was observed with increasing incubation period clearly suggesting the enzyme's role as a primary metabolite, being produced in the log phase of the growth of the fungus for utilization of nutrients (proteins) present in the solid substrate. [12]. The subsequent decrease in the enzyme units could probably be due to inactivation of the enzyme by other constituent proteases. Bacterial DNA content increased with increase in incubation period similar to protease production suggesting that enzyme production was growth associated in nature. These results are in accordance with observations made by Durham *et al*., [13], Gessesse [14] and Yeoman and Edward [15].

Effect of Incubation Temperature: Fermentation carried out at 35°C was best suited for enzyme production. In this study the maximum activity was found at 35°C in all varieties of rice broken (Fig. 4). Preliminary studies on growth and enzyme production at 25, 28 and 32°C indicated that although luxuriant growth occurred at all of these temperatures but the productivity was low at 25°C and higher at 28 and 32°C [16].

Effect of pH: Protease production by microbial strains depends on the extra-cellular pH because culture pH strongly influences many enzymatic processes and transport of various components across the cell membranes, which in turn support the cell growth and product production [17]. The optimum pH for production of protease was recorded at 7.0.in all varieties of rice broken (Fig.5). A notable decline in the enzyme productivity occurred at both higher and lower pH values.Similar results were also reported by Teufel and Gotz [18] that a neutral metalloprotease from *Staphylococcus epidermidis* has pH optimal in the range 5.0-7.0

CONCLUSION

On the light of the obtained results, it could be concluded that fermented PONNI rice broken by the fungus *Aspergillus niger* at 35°C for 72 h and pH 7.0 are the most suitable conditions for protease production.

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