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Enhanced production of cellulases by *Penicillium citrinum* in solid state fermentation of cellulosic residue

R.C. Kuhad* and A. Singh

***Penicillium citrinum*, using rice husks in a solid state fermentation, produced maximum cellulase yields (37 Units/g) after 12 days with a cellulose utilization of more than 70%. Enzyme yields were three times higher than in shake-flask cultures.**

Key words: Cellulase, cellulose, *Penicillium citrinum*, protein, solid state fermentation.

The objective of the present study was to evaluate the potential of a cellulolytic strain of *Penicillium citrinum* for the production of cellulase enzymes and for upgrading rice husk, a major rice byproduct, in solid state fermentation, which has a number of advantages over submerged culture systems (Hesseltine 1972).

Materials and Methods

Microorganism
Penicillium citrinum, isolated locally from decomposed substrates, was grown initially in glucose/yeast extract/broth as described earlier (Singh *et al.* 1988).

Pretreatment of Substrate
Rice husks were washed thoroughly with water to remove surface dust and then dried at 65°C. The dried substrate was autoclaved for 1 h at 121°C with 5% (w/v) NaOH (20 ml per g substrate) and then filtered through muslin cloth. The residue was washed thoroughly with water and neutralized with dilute HCl. The substrate was finally washed with distilled water and dried at 65°C.

Fermentations
Shake-flask cultures were carried out in 250-ml Erlenmeyer flasks using 4% (w/v) substrate in basal medium as described earlier (Singh *et al.* 1988). Basal medium contained (g/l): KH_2PO_4, 10.0; $(NH_4)_2SO_4$, 10.5; $MgSO_4.7H_2O$, 0.3; $CaCl_2$, 0.5; $FeSO_4.7H_2O$, 0.013; $MnSO_4.H_2O$, 0.004; $ZnSO_4.7H_2O$, 0.004; $CoCl_2.6H_2O$, 0.0067; and yeast extract, 0.5 with the pH at 5.0.

R.C. Kuhad is with the Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi-110 021, India. A. Singh is with the Biochemical Engineering Research Centre, I.I.T., Hauz Khas, New Delhi-110 016, India. * Corresponding author.

For solid state culture, 4 g substrate in a 250-ml Erlenmeyer flask was moistened with 20 ml of basal medium and sterilized by autoclaving at 121°C for 30 min. The flasks were inoculated with 5 ml of mycelial suspension and incubated unshaken for 12 days at 28°C. Each experiment was done with triplicate flasks. To extract extracellular enzymes, 30 ml of 50 mM citrate buffer (pH 5.0) was added to the flasks and shaken for 2 h (Singh *et al.* 1988). Thereafter, the contents were filtered and processed as described above.

Analytical Methods
Cellulase activity against filter paper (FPCase), carboxymethyl cellulose (CMCase) and cellobiose (cellobiase) were measured as described by Ghose (1987). Reducing sugars released were determined by the dinitrosalicylic acid method (Miller 1959). One unit of enzyme activity is defined as 1 μmol glucose released/min. ml of culture supernatant. Enzyme production is expressed as units of enzyme per g of original substrate. Total nitrogen content of biomass was analysed by a conventional Kjeldahl method and crude protein was calculated by multiplying the total nitrogen by 6.25. Measurement of cellulose was by the method of Updegraff (1969).

Results and Discussion

Visible observations on the growth of *P. citrinum* in media containing rice husk as sole carbon source in submerged fermentation revealed moderate to dense growth of the culture (Table 1). The culture also effectively colonized the surfaces of husks in solid state fermentation and exhibited dense growth on the alkali-treated substrate. The higher biomass obtained on treated substrate may be explained by the role of NaOH in dissolving lignin and disrupting the

Table 1. Substrate degradation and cellulase and protein production by *P. citrinum* in submerged and solid state fermentations of rice husk.*

Fermentation type	Treatment	Visible growth	Dry weight loss (%)	FPCase (U/g)	Crude protein (%)
Submerged†	Untreated	Moderate	39	5.5	11
	Alkali-treated	Dense	68	10.0	22
Solid state‡	Untreated	Moderate to dense	39	15.3	8
	Alkali-treated	Dense	65	36.9	17

* Data presented are the mean values from three replicates.
† Maximum yields obtained after 7 days in shake-flask culture.
‡ Maximum yields obtained after 12 days.

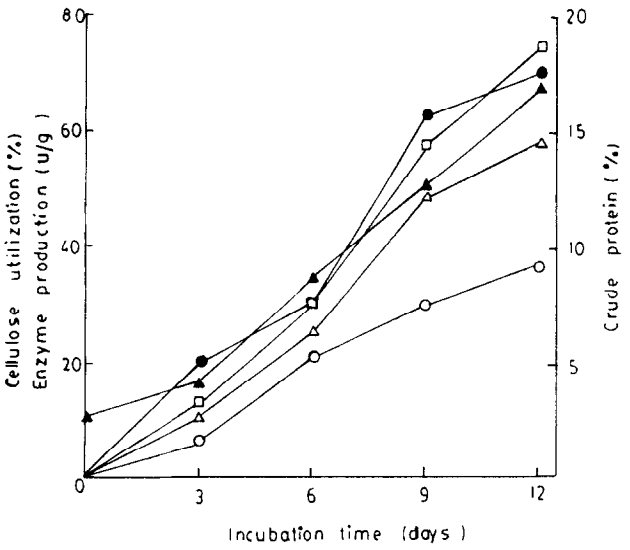

Figure 1. Production of FPCase (○), CMCase (△), cellobiase (□) and protein (▲) and utilization of cellulose (●) by *P. citrinum* in solid state fermentation of alkali-treated rice husk.

association between lignin and cellulose, loosening the crystalline regions of cellulose and thereby enhancing the susceptibility of the substrate to microbial/enzymatic attack.

Although no significant difference in dry weight loss was observed between the submerged and solid state fermentations of rice husk, there were considerable differences in cellulase and protein yields. (Table 1). As expected, enzyme and protein yields were poor with untreated substrates. A two- to 2.5-fold increase in enzyme yield was obtained in both types of fermentations when alkali-treated instead of untreated substrate was used. Interestingly, three-fold higher yields were obtained in solid state cultures of *P. citrinum* compared with those of submerged fermentations.

Figure 1 shows the effect of incubation time on cellulose utilization and cellulase and protein production by *P. citrinum* in solid state fermentations of rice husk. All the three components of cellulase, FPCase, CMCase and cellobiase, showed a similar pattern of enhanced enzyme yield with increased fermentation time. Maximum FPCase (36.9 U/g), CMCase (58.8 U/g) and cellobiase (75.2 U/g) activities were obtained after 12 days. Production of protein progressively increased up to 12 days of fermentation and thereafter remained more or less constant. A similar trend of cellulose utilization was obtained, suggesting a positive correlation between cellulose utilization and protein production by *P. citrinum*.

Our results indicate the potential of *P. citrinum* in producing high yields of cellulase enzyme in solid state fermentations of cellulosic substrates. Further optimization of cultural and nutritional factors may result in better cell growth and enzyme yields.

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