

Full Length Research Paper

Enhanced production of cellulases by various fungal cultures in solid state fermentation of cassava waste

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Accepted 27 February, 2006

Cellulases are a group of hydrolytic enzymes capable of degrading cellulose to the smaller glucose units. These enzymes are produced by fungi and bacteria. The solid waste of sago industry using cassava tubers was fermented by *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus stolonifer* in solid state fermentation. The cassava waste contained dry wt of 13.4% cellulose and 2.9% protein by dry weight. The highest cellulase activity was observed on the 10th day in *R. stolonifer* mediated fermentation. *R. stolonifer* was more efficient in bioconverting cassava waste into fungal protein (9%) compared to *A. niger* and *A. terreus*.

Key words: *Aspergillus niger*, *Aspergillus terreus*, *Rhizopus stolonifer*, cellulase, solid state fermentation, cassava waste.

INTRODUCTION

Cellulose, being an abundant and renewable resource, is a potential raw material for the microbial production of food, fuel and chemicals (Coughlan, 1985). Various bacteria, actinomycetes and filamentous fungi produce extra cellular cellulases when grown on cellulosic substrates though many actinomycetes have been reported to have less cellulase activity than moulds (Ishaque and Kluepfel, 1980; Kluepfel et al., 1986). Investigations on the extracellular cellulases of fungi have been concentrated mainly on *Trichoderma* sp. (Jabbar and Ilahi, 1981; Ross et al., 1983; Ghosh et al., 1984) and studies on other mesophilic fungi suggested the possibility that other cellulase systems could be utilized for the hydrolysis of cellulose (Singh et al., 1988; Darmwal et al., 1988).

The presence of lignin in cellulosic substrates and the crystalline nature of cellulose make it inaccessible to cellulase. Cellulase is a complex enzyme having chiefly endo and exo-1,4 glucanase and β -glucosidase activities. A synergistic action of these enzymes is required for the complete hydrolysis of cellulose. The objective of the present study was to evaluate the potential of the cellulolytic strains of *Rhizopus stolonifer*, *Aspergillus*

niger and *Aspergillus terreus* for the production of cellulase enzymes and for upgrading cassava waste in solid state fermentation.

MATERIALS AND METHODS

Substrate

Fresh cassava waste was collected from Varalakshmi Sago industry, Namagiri, Salem (Dt), India. It was sun dried, coarsely ground to uniform size (5 mm) and stored in gunny bags and was used within one month after procurement.

Microorganisms

Rhizopus stolonifer, *Aspergillus niger* and *Aspergillus terreus* were isolated by primary selection from a naturally contaminated cassava waste by serial dilution and pour plate technique. The pure cultures were identified by their morphology and colony characteristics. The organisms were maintained on PDA slants and stored at 4°C. The slants were freshly made once a month.

Solid state fermentation

Solid state fermentation of cassava waste was carried out in 250 ml Erlenmeyer flasks. Twenty gram of cassava waste was taken in individual flasks and 60 ml distilled water was added to give

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Table 1. Cellulose content (mg/g) of cassava waste during solid state fermentation with selected fungi.

Organisms	Cellulose content (mg/g)					
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
<i>Rhizopus stolonifer</i>	134.28±11.08	46.15±2.01	40.32±1.17	34.06±0.71	26.8±0.71	8.24±0.10
<i>Aspergillus niger</i>	134.28±11.08	79.53±6.51	40.32±1.09	28.49±1.46	14.90±0.66	14.72±0.76
<i>Aspergillus terreus</i>	134.28±11.08	83.80±6.37	42.19±1.33	16.68±1.87	15.63±1.01	15.07±1.01

Results are mean ± SE of three replicates.

Table 2. Production of CMCase, FPase, β-glucosidase, protein and utilization of cellulose by *Rhizopus stolonifer* in solid state fermentation of cassava waste.

Parameters	Incubation time (days)					
	0	2	4	6	8	10
CMCase (IU/ml)	ND	0.44±0.01	0.34±0.02	0.026±0.01	0.31±0.03	0.46±0.03
FPase (IU/ml)	ND	0.28±0.01	0.20±0.02	0.13±0.01	0.23±0.01	0.26±0.02
β-Glucosidase (IU/ml)	ND	0.38±0.01	0.32±0.01	0.25±0.01	0.38±0.02	0.41±0.02
Protein (%)	2.9±0.26	5.6±0.26	7.2±0.24	8.9±0.28	9.0±0.43	8.2±0.28
Cellulose utilization (%)	ND	65.6±1.82	69.9±2.14	74.6±3.17	80.4±4.52	93.8±4.81

Results are mean ± SE of three replicates. ND: Not detected.

moisture content of 75%. The flasks were plugged with cotton and autoclaved at 121°C for 15 min. Agar blocks (8 mm disc) removed from the plates containing seven days old cultures of fungi were used as inoculums for solid state fermentation. A single block was aseptically inoculated into each flask containing the substrate. Three replicates were maintained for each organism. The flasks were incubated at room temperature for ten days. Bioconverted cassava waste samples were withdrawn at intervals of two days, oven dried at 60°C and were analyzed for cellulose and protein. The activity of the enzyme cellulases was assayed using fresh fermented sample without drying during the course of fermentation.

Analytical methods

Protein content was estimated by Lowry et al. (1951). Cellulose was quantified by the method of Updegraff (1969). Cellulase activity against filter paper (FPase), carboxymethyl cellulase (CMCase) and β-glucosidase were assayed by Ray et al. (1993) method. 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.

RESULTS AND DISCUSSION

Table 1 shows that the rate of utilization of cellulose in cassava waste by *R. stolonifer* was very rapid, with 66% of the cellulose being utilized within the first two days of inoculation. There after the rate of utilization of cellulose was steady till the sixth day and by the tenth day 94% of cellulose had been utilized. While *A. niger* utilized 89% cellulose in 8 days; nearly the same amount (88%) was utilized in six days by *A. terreus* after which there was no further increase in the rate of hydrolysis by both the organisms. The cellulose degrading potential of *A. terreus* was confirmed by Ali et al. (1991) and Szakacs et al. (2001). The protein content of the cassava waste

increased by 2.13 fold due to the growth of *R. stolonifer*. *Aspergillus niger* and *A. terreus* brought about a maximum 1.8 and 1.62 fold increase in protein content on the 8th and 4th day, respectively (Tables 2, 3 and 4). A similar trend of cellulose utilization was obtained, suggesting a positive correlation between cellulose utilization and protein production by selected fungi. These results are in conformity with those of Kuhad and Singh (1993) who observed a 2.5 fold increase in protein in a cellulosic residue using *Penicillium citrinum*.

Tables 2 to 4 shows the CMCase activity of *R. stolonifer* declined from second day till the sixth day of fermentation and the activity again increase till the final day of fermentation. The peak activity was observed in the tenth day 0.44 IU/ml. CMCase activity of *A. niger* and *A. terreus* built up slowly to reach the maximum activity on the 8th day fermentation (0.12 and 0.1 IU/ml, respectively). CMCase activity of both of these organisms was significantly lower than that of *R. stolonifer* at all the days of fermentation. Elshafei et al. (1990) compared the cellulase and hemicellulase activity of sixteen fungi utilizing corn stover as the substrate and they reported that *A. terreus* showed a higher activity for all three of the cellulolytic enzyme assays (CMCase, FPase and β-glucosidase) than *Trichoderma viride*, which is considered as the standard for cellulase and hemicellulase activity. In the present investigation *A. terreus* showed comparatively lower cellulolytic enzyme activity than *R. stolonifer* and *A. niger*. But direct comparison of these results would be difficult, since many factors including media composition and choice of substrate affected enzyme activity (Sharma et al., 1986). The higher cellulase activities of *R. stolonifer* might be due to the good growth of its mycelial biomass in cassava waste, which led to higher

Table 3. Production of CMCase, FPase, β -glucosidase, protein and utilization of cellulose by *Aspergillus niger* in solid state fermentation of cassava waste.

Parameters	Incubation time (days)					
	0	2	4	6	8	10
CMCase (IU/ml)	ND	0.02±0.001	0.03±0.001	0.04±0.002	0.12±0.002	0.07±0.002
FPase (IU/ml)	ND	0.008±0.001	0.01±0.001	0.02±0.001	0.04±0.001	0.03±0.001
β -Glucosidase (IU/ml)	ND	0.06±0.001	0.07±0.002	0.1±0.002	0.1±0.001	0.08±0.001
Protein (%)	2.9±0.26	5.6±0.28	6.7±0.21	7.4±0.18	8.1±0.44	7.8±0.68
Cellulose utilization (%)	ND	40.7±2.08	69.9±2.84	78.8±3.17	88.9±3.96	89.0±4.21

Results are mean \pm SE of three replicates.
ND: Not detected.

Table 4. Production of CMCase, FPase, β -glucosidase, protein and utilization of cellulose by *Aspergillus terreus* in solid state fermentation of cassava waste.

Parameters	Incubation time (days)					
	0	2	4	6	8	10
CMCase (IU/ml)	ND	0.03±0.001	0.04±0.001	0.05±0.001	0.1±0.003	0.08±0.001
FPase (IU/ml)	ND	0.007±0.01	0.009±0.01	0.03±0.001	0.04±0.001	0.03±0.001
β -Glucosidase (IU/ml)	ND	0.06±0.001	0.07±0.001	0.07±0.002	0.11±0.002	0.07±0.001
Protein (%)	2.9±0.26	4.5±0.32	7.6±0.4	5.5±0.28	4.5±0.19	4.4±0.18
Cellulose utilization (%)	ND	37.5±1.72	68.6±2.41	87.6±2.53	88.4±3.24	88.7±3.18

Results are mean \pm SE of three replicates.
ND: Not detected.

enzyme production. Similar observations have been expressed by Vipani et al. (1994). Cellulase production from various *Aspergillus* strains has been studied either on semi solid media or under submerged conditions (Coral et al., 2002; Onsoni et al., 2005). *A. niger* has been used mostly for the production of cellulase (Ray et al., 1993). However, in the present investigation the cellulase enzyme activity of *A. niger* was observed to be surpassed by the activities of *R. stolonifer*.

Ali et al. (1991) reported a maximum yield of cellulase from *A. terreus* at 40°C on water hyacinth after 6 days. The maximum cellulase activity of *A. terreus* in this experiment was observed on the 8th day. The higher relative activity of cellulase from *A. terreus* has been reported in many cellulosic substrates (Vanwyk, 1998). The activities of cellulase enzyme complex of *R. stolonifer*, *A. niger* and *A. terreus* did not corroborate with the activities of *T. viride* and *Sclerotium rolfsii* reported by Vipani et al. (1994). But the results for *A. niger* conformed with those of Lakshmikanth (1990).

Maximum activities of cellulase were obtained on the eighth day in *A. niger* and in *A. terreus* fermentation, and on the tenth day in *R. stolonifer* fermentation of cassava waste. Ray et al. (1993) reported maximum production of cellulases on the 15th day of fermentation on wheat bran by *A. niger*. Fpase and β -glucosidase of all the three organisms showed a pattern similar to that of their respective CMCase activity. Enzyme extracts obtained from *R. stolonifer*, *A. niger* and *A. terreus* were found to

be rich in β -glucosidase than FPase. This is of importance because low level of β -glucosidase in cellulose system results in the accumulation of cellobiose which decreases the rate and extension of cellulose hydrolysis (Lakshmikanth, 1990; Persson et al., 1991; Ray et al., 1993).

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