Mycobiology 34(3): 128-130 (2006)
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Raw Starch Degrading Amylase Production by Various Fungal Cultures Grown on Cassava Waste

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(Received July 10, 2006)

The solid waste of sago industry using cassava was fermented by *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus stolonifer* in solid state fermentation. Cassava waste contained 52 per cent starch and 2.9 per cent protein by dry weight. The amylase activity was maintained at a high level and the highest amylase activity was observed on the 8th day in *R. stolonifer* mediated fermentation. *R. stolonifer* was more efficient than *Aspergillus niger* and *Aspergillus terreus* in bioconverting cassava waste into fungal protein (90.24 mg/g) by saccharifying 70% starch and releasing 44.5% reducing sugars in eight days of solid state fermentation.

KEYWORDS: *Aspergillus niger*, *Aspergillus terreus*, Cassava waste, *Rhizopus stolonifer*, Solid state fermentation

Large quantities of starchy or lignocellulosic agro-industrial wastes and crop residues are made available every year in many tropical countries, posing severe environmental pollution problems. Efficient and controlled biodegradation of these materials by fungi or bacteria leads to a number of processes of great economic importance (Ray *et al.*, 1993).

Starch is a promising substrate for the production of glucose, fuels and single cell protein (SCP). Various amylolytic fungi have been used for the production of SCP and amylase from starchy materials in submerged agitated culture (Kallel-Mhiri, 1994; Soccol, 1994). Several workers, however, have turned their attention to biodegradation of starch using solid state fermentation because this method has been found to be the more appropriate system than submerged fermentation for protein enrichment and amylase production from starchy materials (Park and Rivera, 1982; Forgarty, 1983; Illanes, 1983; Saha and Zeikus, 1989; Pandey *et al.*, 2001).

We have earlier reported the conversion of cellulose and starch using various fungi under solid state fermentation (Eyini *et al.*, 2002). Cassava is one of the major cash crops in India. Large quantities of tippi are disposed off from sago processing industries. The present investigation reports the potential of starch utilization and protein productivity of three amylolytic fungi during solid state fermentation of cassava waste.

Materials and Methods

Aspergillus niger, *Aspergillus terreus* and *Rhizopus stolonifer* were isolated from naturally contaminated cassava waste by enrichment culture technique. All the three fungi were maintained on potato dextrose agar slant, stored at 4°C and were subcultured once a month.

The fermentation processes were carried out in 250 *ml* Erlenmeyer flasks with 20 g cassava waste containing 70 percent moisture. The flasks were plugged with cotton and sterilized at 121°C and 15 pounds pressure for 15min. Two agar blocks (8 mm discs) from actively growing, 7 days-old plates of fungal pure cultures were inoculated into the flasks and they were incubated as static cultures at room temperature (28 ± 2°C) for ten days. Composition of cassava waste was analyzed for starch, reducing sugars, and protein. The contents of the flasks were removed periodically at an interval of two days from the second day of solid state fermentation and were analyzed for starch (Arditti and Dunn, 1969), reducing sugars (Miller, 1959) and protein (Lowry *et al.*, 1951). Amylase activity (Miller, 1959) was assayed in the fermented sample at an interval of two days. Amylolytic enzyme activities were expressed in international units (IU/*ml*) defined as the micromoles of glucose liberated by 1 *ml* of enzyme in 1min. The experiments were carried out with three replicate samples.

Results and Discussion

Cassava waste contained 52 percent starch, 2.9 per cent protein and 1.4 percent free reducing sugars. Table 1 showed 51 percent utilization of starch in the substrate by *R. stolonifer* within two days, while *A. niger* and *A. terreus* had taken four days for utilizing nearly the same amount of starch (55 percent and 52 percent starch utiliza-

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tion respectively). The better performance of *R. stolonifer*, which showed higher and faster utilization of starch than *A. niger* and *A. terreus* indicated its higher potential for starch degradation. The starch utilizing potential of *R. stolonifer* and *A. niger* had been well documented (Saha and Zeikus, 1989; Hayashida *et al.*, 1988; Kim *et al.*, 1989).

R. stolonifer grown on tippi, produced the maximum mycelial protein, 9 percent, on the eighth day of fermentation. The maximum biomass protein produced by *A. niger* and *A. terreus* was comparatively less (8.1 per cent and 7.6 per cent, respectively). *A. terreus* attained the maximum biomass in terms of mycelial protein on the fourth day of fermentation, four days earlier than other two fungi. After the day of peak in mycelial protein production, a gradual decrease in protein content was ob- served in the solid state fermentation (Table 1). Similarly accumulation of reducing sugars has been observed to inhibit cell density or biomass (Tabassum *et al.*, 1990). Protein productivity (g protein produced/g starch consumed) of *R. stolonifer*, *A. niger* and *A. terreus* was 0.17, 0.15, and 0.14, respectively, (Table 1). Zabala *et al.* (1997) had shown that protein productivity was negatively correlated with the progress of solid state fermentation process in *Trichoderma reesei* which showed high protein productivity (0.33) in the substrate.

The percent saccharification of the substrate was increased by *R. stolonifer* and *A. niger* (75 per cent and 68.5 respectively) and the protein content increased from 2.9 percent to 9 percent and 8 percent respectively, on the eighth day of solid state fermentation of tippi. *A. terreus* increased the substrate protein content by 7.6 percent and it could saccharify only 67 percent of the substrate (Table 1).

The yield co-efficient between consumed starch and synthesized proteins on the eighth day of SSF of cassava waste by *R. stolonifer* was 0.25, which was higher than the other two selected fungi. Soccol *et al.* (1995), using a spore suspension inoculum of *R. stolonifer*, obtained a high yield co-efficient of 0.5 during SSF of cassava bagasse.

The production of reducing sugars in cassava waste during the solid state fermentation process was more in *R. stolonifer* (44.5 per cent) than in *A. niger* and *A. terreus* (20.2 per cent and 18.9 per cent) mediated fermentation. *R. stolonifer*, giving a higher yield of reducing sugars, was also observed to have higher cassava utilizing potential (Table 2). Similar results have been reported earlier with studies on cassava bagasse waste (Carta *et al.*, 1997).

Starch utilization potential of these fungi can be correlated with the activities of starch saccharifying enzymes *viz* amylase and amylogluosidase. The successful degradation of cassava waste by *R. stolonifer* could be attributed to its higher amylolytic potential. Similar results have been reported earlier with studies on *A. niger* and other fungi grown on spent grain liquor (Akpan *et al.*, 1999), spent grains (Ofuya *et al.*, 1989), and cassava peel (Ofuya and Nwajiuba, 1990).

Table 1. Starch and protein content (%) of cassava waste during solid state fermentation with selected fungi*

Organisms	Starch (%)							Protein (%)						
	Fermentation period (Days)						a	Fermentation period (Days)						b
	0	2	4	6	8	10		0	2	4	6	8	10	
Rhizopus stolonifer		25.6±0.8	18.9±1.4	17.2±0.5	15.7±1.3	13.0±0.5	75.0±3.7		5.6±0.2	7.2±0.2	8.9±0.2	9.0±0.4	8.2±0.2	0.17±0.005
Aspergillus niger	52.0±0.9	33.5±2.1	23.5±0.8	19.7±0.7	18.5±0.7	16.4±0.6	68.5±2.8	2.9±0.2	5.5±0.2	6.7±0.1	7.4±0.1	8.1±0.4	7.8±0.6	0.15±0.006
Aspergillus terreus		33.1±1.0	24.8±1.0	20.3±1.2	18.6±0.7	17.2±1.3	67±2.1		4.5±0.3	7.6±0.6	5.5±0.2	4.5±0.1	4.4±0.1	0.14±0.002

* Results are mean ± standard error of three replicates.
a, Percent saccharification.
b, Protein productivity.

Table 2. Reducing sugars (%) of cassava waste during solid state fermentation with selected fungi*

Organisms	Reducing sugars (%)					
	Fermentation period (Days)					
	0	2	4	6	8	10
Rhizopus stolonifer		25.3±1.4	27.8±1.7	31.2±0.7	44.5±1.5	20.3±1.1
Aspergillus niger	1.4±1.1	6.7±0.7	12.1±0.8	14.4±0.9	20.2±1.6	12.3±0.6
Aspergillus terreus		6.4±0.4	12.5±0.9	18.9±1.4	9.7±0.3	9.5±0.4

*Results are mean ± standard error of three replicates.

Table 3. Amylase activity (IU/*ml*) of selected fungi during solid state fermentation*

Organisms	Amylase activity (IU/*ml*)				
	Fermentation period (Days)				
	2	4	6	8	10
Rhizopus stolonifer	0.85±0.01	0.85±0.02	0.85±0.01	1.0±0.04	0.25±0.02
Aspergillus niger	0.55±0.01	0.64±0.02	0.68±0.03	0.11±0.02	0
Aspergillus terreus	0.52±0.03	0.60±0.02	0.74±0.03	0.38±0.02	0

*Results are mean ± standard error of three replicates.

Results of amylase assay (Table 3) in this study also showed higher activity of amylase in *R. stolonifer* compared to other two organisms. The highest amylase activity (1 IU/*ml*) was observed on the eighth day in *R. stolonifer* mediated fermentation, while the amylases of *A. niger* and *A. terreus* showed the maximum activity (0.68 and 0.74 IU/*ml* respectively) on the sixth day of fermentation. The activity could not be detected on the final day in the culture filtrate of *A. niger* and *A. terreus*.

Amylase activity increased in proportion with an increase in biomass or mycelial protein in *A. niger* and *A. terreus*, while in *R. stolonifer*, a high level of amylase activity was maintained for the most part and the peak amylase activity was observed on eigth day of SSF.

The increase in starch saccharification of the cassava waste substrate fermented by the fungal cultures may be attributed to the significant increase in activities of amylase enzyme complex including amylase and amylo glucosidase. Forgarty (1983) and Soccol *et al.* (1994) observed similarly that complete amylase production was essential for efficient starch degradation. Further work on the amylo glucosidase activity of the fungal cultures and the effect of carbon and nitrogen supplementation on the enzyme activities during SSF on cassava waste is in progress.

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