Cellulose Utilization and Protein Productivity of Some Cellulolytic Fungal Co-cultures

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Protein productivity by the cellulolytic fungi, Trichoderma viride (MTCC 800), Chaetomium globosum and Aspergillus terreus was compared in co-culture and mixed culture fermentations of cashewnut bran. Co-cultures were more effective in substrate saccharification, which ranged between 85~88% compared to the 62~67% saccharification shown by the monocultures. Maximum saccharification was induced by T. viride and C. globosum co-culture resulting in the highest 34% release of reducing sugars. The maximum 16.4% biomass protein and the highest protein productivity (0.58%) were shown by T. viride and A. terreus co-culture. A. terreus performed better in co-culture in the presence of T. viride rather than with C. globosum. Among the cellulolytic enzymes, FPase (Filter Paper Cellulase) activity was significantly higher in all the co-cultures and in the mixed culture than in their respective monocultures. Mixed culture fermentation involving all the three fungi was not effective in increasing the per cent saccharification or the biomass protein content over the co-cultures.

KEYWORDS: Aspergillus terreus, Chaetomium globosum, CMCase, FPase, β-glucosidase, Trichoderma viride

Large quantities of cellulosic 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).

To improve the conversion of cellulosic biomass to chemicals and fuels, many hyper cellulolytic strains have been used either as pure cultures or as mixed cultures with fermenting organisms (Lezinou et al., 1995; Tanaka et al., 1986). The use of mixed cultures of lignocellulolytic or cellulolytic microorganisms looks promising in increasing the protein content compared to pure cultures (Rabala et al., 1994) and many of them have been reported to be more efficient in degrading lignocellulosic substrates and in producing high activity enzymes than the monocultures (Arora, 1995; Puniya and Singh, 1995).

One important aspect of cellulose research using SSF (Simultaneous Saccharification Fermentation) has been on co-culturing of two cultures together for enhanced enzyme production. A co-culture of Aspergillus ellipiticus and A. fumigatus resulted in improved hydrolytic and β-glucosidase activities as compared to the occasions when they were used separately (Tengerdy, 1996).

Synergism between individual components of cellulase from different origins on substrates such as cotton (Sadama and Patil, 1985) and different paper products (van Wyk, 1998), has been applied with varying degrees of success. Solid state fermentation has been found to be the more appropriate system than submerged fermentation for protein enrichment and cellulase production from lignocellulose (Elshafei et al., 1990; Illanes et al., 1998; Pandey et al., 2001).

Cashewnut is one of the major cash crops in India. Cashewnut bran represents 1.5% of the dry weight of whole nut. Large quantities of cashewnut bran are disposed off from cashewnut processing industries. The present investigation reports the potential of cellulose utilization and protein productivity of three cellulosic fungi during co-culture and mixed culture fermentation of cashewnut bran.

Materials and Methods

Trichoderma viride (MTCC 800) strain was procured from National Chemical Laboratory, Pune, India. Aspergillus terreus and Chaetomium globosum were isolated from naturally contaminated cassava waste and from the effluent of a paper mill, respectively 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 cashewnut bran (20 g) containing 70% moisture. The flasks were plugged with cotton and sterilized at 121°C and 15 pounds pressure for 15 min. Two agar blocks (7 mm each) from actively growing 7 day-old plates of fungal pure cultures were inoculated into each flask for monoculture fermentation. For co-cul-
structure fermentations, a single agar block from each of the two fungi in the three permutation and combination was inoculated into each flask. In mixed culture studies, a single agar block from each of the three fungal cultures, T. viride, A. terreus and C. globosum was inoculated into each conical flask.

All the flasks were incubated as static cultures at room temperature for 25 days. Composition of cashewnut bran was analyzed for cellulose, reducing sugars and protein. The contents of the flasks were removed periodically at an interval often days from the 5th day of SSF (Simultaneous Saccharification Fermentation) and were analyzed for cellulose (Updegraff, 1969) reducing sugars (Maheswari et al., 1993) and protein (Lowry et al., 1951). The activities of enzymes, CMCase (Carboxymethylcellulase), FPase (Filter Paper Cellulase) and β-glucosidase (Ray et al., 1993) were assayed at an interval of five days. All the three cellulolytic enzyme activities were expressed in International Unit (IU/ml). One International Unit is defined as the micromoles of glucose liberated by 1 ml of enzyme in 1 min. The experiments were carried out with three replicate samples.

Results and Discussion

Cashewnut bran contained 32.5% cellulose, 4% protein and 3.3% free reducing sugars. The increase in the protein content (from 4% to 12.1%) and the per cent saccharification (66% and 67% respectively) of the substrate were nearly equal in the monocultures of T. viride and C. globosum on the 25th day of solid state fermentation of cashewnut bran. A. terreus monoculture increased the substrate protein content by 6.8% and it could saccharify only 62% of the substrate (Table 1).

Co-cultures involving these organisms were more effective than their respective monocultures, showing 85~88% saccharification in the same period. Among the co-cultures, A. terreus and C. globosum co-culture was the least effective producing 12.4% biomass protein by saccharifying 85% of the substrate in 25 days (Table 1). The maximum 88% saccharification was observed in co-cultures involving T. viride and C. globosum, resulting in the release of the highest 34% reducing sugars, while the maximum biomass protein was produced in T. viride and A. terreus co-culture (16.4%). In the mixed culture fermentation, more reducing sugars were produced than the co-culture of T. viride and A. terreus, but its biomass production was less than that of the latter. Similarly, accumulation of reducing sugars has been observed to inhibit cell density or biomass (Tabassum et al., 1990). Mixed cultures involving all the three fungi could neither improve the biomass protein nor induce better saccharification than the co-cultures.

Protein productivity (g protein produced/g cellulose consumed) of T. viride, C. globosum and A. terreus monocultures was 0.38, 0.37 and 0.34, respectively (Table 1). These results are in conformity with those of Zabala et al. (1994) who reported a protein productivity value of 0.33 for T. reesei and those of Puniya and Singh (1995) who observed a 4.67% protein enrichment in wheat straw using Phaenerochaete chrysosporium and Azotobacter chroococcum.

Co-cultures involving T. viride as one of the partners showed more synergistic growth (protein productivity 0.55

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organisms</th>
<th>Cellulose (%)</th>
<th>Reducing sugars (%)</th>
<th>Proteins (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>Trichoderma viride</td>
<td>32.5±2.8</td>
<td>23.8±1.6</td>
<td>15.2±1.1</td>
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<td>2</td>
<td>Aspergillus terreus</td>
<td>25.4±1.9</td>
<td>18.1±1.0</td>
<td>12.2±1.0</td>
</tr>
<tr>
<td>3</td>
<td>Chaetomium globosum</td>
<td>21.7±1.2</td>
<td>14.3±1.0</td>
<td>10.7±1.0</td>
</tr>
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<td>15.3±1.0</td>
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<tr>
<td>5</td>
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<td>18.7±1.5</td>
<td>8.2±1.0</td>
<td>4.8±1.0</td>
</tr>
<tr>
<td>6</td>
<td>Coculture 3</td>
<td>16.3±1.1</td>
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</tr>
<tr>
<td>7</td>
<td>Mixed culture</td>
<td>14.9±0.9</td>
<td>8.5±0.4</td>
<td>4.5±0.1</td>
</tr>
</tbody>
</table>

a: Percent saccharification.

b: Protein productivity [protein (g) produced/cellulose (g) utilized].
and 0.58) than the co-culture involving *C. globosum* and *A. terreus*. *A. terreus* monocultures with low protein productivity showed higher rate of cellulose utilization and protein productivity in co-cultures. This may be due to the increased growth of the slow growing organism such as *A. terreus* in the presence of the other member in co-cultures. Similar results were reported by Zabala et al. (1994) in a mixed culture of *T. reesei* and *Monosporium* sp.

The relative activities of the three cellulolytic enzymes varied with the organism employed, the combination of co-culture and the incubation period (Figs. 1, 2 and 3). Among the cellulolytic enzymes, β-glucosidase activity was found to be higher in all the cultures. The co-culture, *A. terreus* with *T. viride* exhibited the maximum β-glucosidase activity on the 25th day of incubation in cashew nut bran. Similarly other lignocellulosic substrates like wheat straw and bagasse have been reported to be the best inducers for β-glucosidase (Lakshmikant and Mathur, 1990).

All the three fungal monocultures showed peak activities of CMCase, FPase and β-glucosidase on the 25th day of fermentation. Similarly Lakshmikant and Mathur (1990) obtained maximum cellulase activity between 16 days and 20 days of incubation using *C. globosum* on various cellulotic substrates.

While CMCase and β-glucosidase activities started dramatically increasing from the 20th and 10th day of SSF respectively, FPase activities were comparatively lower and they increased gradually throughout the experimental period in the monocultures. Even though *A. terreus* grown on corn stover in liquid culture reportedly produced more CMCase, FPase and β-glucosidase earlier than *T. viride* (3), we observed that the production of CMCase and β-glucosidase on cashew nut bran by *A. terreus* was less than that of *T. viride* and *C. globosum*. Direct comparisons of the results obtained in this study with similar results of other researchers are difficult, since many factors, including media composition and choice of substrate affect enzyme activity (Shamala and Sreekantiah, 1986). However similar very low FPase activities as against CMCase and β-glucosidase activities observed in the monocultures in this study were reported by Elshafai et al. (1990).
Gattlen (1981) observed that complete cellulase production was essential for efficient cellulose degradation.

Though the cellulase activities were maximum on the 25th day of SSF in all the monocultures and co-cultures, the rate of cellulose utilization decreased after the 20th day, indicating that there was no relationship between cellulase production and substrate utilization as was observed earlier for T. reesei growing on wheat straw (Maheswari et al., 1993).

Although A. terreus monoculture showed lower relative cellulase activity than the other monocultures, \( \beta \)-glucosidase activity of A. terreus and T. viride co-culture was higher than the other co cultures (1.99 IU/ml). This may explain the higher per cent saccharification (88%) induced by this co-culture. Similar synergism between cellulases of \( \beta \)-glucosidase activity of some cellulosic wastes. World J. Microbiol. Biotechnol. 6: 23-26.


Refesnces


