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Production of Soluble Crude Protein Using Cellulolytic Fungi on Rice Stubble as Substrate under Waste Program Management

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The investigation was undertaken to enhance the decomposition process by pre-treatment of rice stubble, having higher concentration of lignin. Air-dried rice stubble was treated with 1.8 liter of 1% NaOH and autoclaved. Six cellulolytic fungi, *Trichoderma harzianum*, *Penicillium citrinum*, *Curvularia lunata*, *Aspergillus flavus* and *Alternaria alternata* were grown in basal synthetic medium along with delignified rice-residue as carbon source for production of soluble crude protein. Though the loss of cellulose has been observed by all of them but having a considerable status in the presence of *T. harzianum* and *T. harzianum* yielded highest percentage of crude protein (27.99%) with biomass of 375 mg, whereas the lowest protein value (17.91%) was recorded in case of *A. niger* with biomass of 422 mg. Among the imperfect fungi, *T. harzianum* was the most potent. Effects of incubation period and nitrogen sources on soluble crude protein production by *T. harzianum* were also undertaken in this study. Fifth day of incubation period and potassium nitrate as nitrogen source among other nitrogen sources was found most appropriate for soluble crude protein production by the mentioned organism.

KEYWORDS: Decomposition, Rice stubble, Soluble crude protein (SCP).

With the introduction of new varieties, modern techniques and more area under irrigation have increased the rice production substantially. Mechanization of harvesting through harvester has created a problem of waste management. Presently, this waste is disposed off through burning, which creates environmental problem by way of air pollution, and disturbes the rich soil biodiversity through heating. Rice stubble contains considerable amount of cellulose and lignin because its decomposition takes longer time. Dhillon *et al.* (1980) used eight cellulolytic fungi for production of soluble crude protein from delignified rice straw using *Chaetomium globosum* as highest soluble crude protein producer. *C. thermophile* was efficient soluble crude protein producer when grown on delignified wheat straw (Sekhon, 1975). During decomposition proportion of mannose, galactose, fructose, rhamnose and ribose increased consistently with time, whereas proportion of cellulolysic glucose decrease (Murayama, 1984). In this study, rice stubble on which very scanty work seems to be done is taken as a material for soluble crude protein production using cellulolytic fungi.

Materials and Methods

Strains, media and culture conditions. The six cellulolytic fungi, *Trichoderma harzianum*, *Penicillium citrinum*, *Curvularia lunata*, *Aspergillus flavus* and *Alternaria alternata* were grown in basal synthetic medium described by Chahal and Gray (1969). One hundred grams of air dried rice stubble, 2~3 cm in length were autoclaved with 1.8 litres of 1% sodium hydroxide at 121°C under 15 lbs for one hour.

Estimation of soluble crude protein production. The procedure for estimation of soluble crude protein production by these six cellulolytic fungi was used as suggested by Dhillon *et al.* (1980). After squeezing through nylon cloth the stubble was thoroughly washed with distilled water till neutral and dried at 60°C. The pre-treated and dried rice straw ground to 60-mesh was used as sole source of carbon. Four 250 *ml* Erlenmeyer flasks containing 50 *ml* basal synthetic medium along with fungal culture and 500 mg delignified rice residue were incubated on a rotary shaker at 28 ± 1°C. Five days after incubation the contents of the flasks were filtered through tared Whatman filter No. 1 to determine the weight of the fungal mycelia and undigested cellulosic materials. The dried biomass was analyzed for its nitrogen content through Kjeldhal method (Jackson, 1973). To get the protein value, this nitrogen content is multiplied by a constant factor 6.24. *T. harzianum* was incubated separately on basal synthetic medium for eight days to find the effective day of incubation on SCP production. We tried eight different sources, ammonium nitrate, ammonium chloride, ammonium sulphate, ammonium dihydrogen phosphate, diammonium hydrogen phosphate, potassium nitrate, sodium nitrate and urea to find out the most suitable nitrogen source of SCP production. The amount of nitrogen

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Table 1. Single cell protein production by different cellulolytic fungi

Fungi	Biomass (Mycelium + undigested rice-residue) mg	Crude protein (%)	Relative protein production efficiency (%)
Trichoderma harzianum	375	27.99	100.0
Pencillium critinum	390	24.96	89.0
Curvularia lunata	400	23.34	83.4
Aspergillus flavus	403	21.67	77.4
Alternaria alternata	405	20.82	74.4
Aspergillus niger	422	17.91	64.0
SEm ±		0.24	
CD (P = 0.05)		0.71	

added to medium was equivalent to 400 mg of nitrogen/ litre of medium.

Results and Discussion

The data shown in Table 1 reveals that six cellulolytic fungi tested on delignified cellulose as a carbon source varied widely in soluble crude protein (SCP) production. *T. harzianum* resulted in the highest SCP production which was significantly superior to other fungi. *P. citrinum* and *C. lunata* brought about significant increase in SCP compared to the rest of the fungi but varied significantly between themselves. The former proved significantly superior to the latter. Differences among *A. flavus*, *A. alternata* and *A. niger* were significant. The latter species proved significantly inferior to the former two species in producing SCP. The SCP production efficiency of cellulolytic fungi was in order of *T. harzianum* > *P. citrinum* > *C. lunata* > *A. flavus* > *A. alternata* > *A. niger*. Similar results were earlier reported by Chahal and Gray (1969) and Dhillon *et al.* (1980). Fungi have the ability to produce a variety of enzymes. *A. niger*, *A. flavus* and *Penicillium* spp. have been reported to be main sources of cellulase, amylase, hemicellulase, catalase, pectinase and xylanase (Hamlyn, 1998).

Fungi also varied in biomass production. The maximum biomass production was achieved by *A. niger* and minimum by *T. harzianum* but biomass weight loss was reverse. This is consistent with the findings of Chahal and Gray (1969) and Hobbie *et al.* (2003).

Table 2. Effect of incubation period on the SCP production by *Trichoderma harzianum*

Days	Dry weight (Mycelium + undigested rice residue)	Protein %
1	382	4.7
2	385	8.0
3	355	16.0
4	307	23.0
5	390	28.0
6	380	27.0
7	382	26.5

Rapid and higher production of SCP by fungi from delignified cellulose may be ascribed to increased availability of amorphous form of cellulose owing to delignification with sodium hydroxide. Punj *et al.* (1971) from their detailed studies of decomposition of delignified cellulose reported that delignification of residue with sodium hydroxide results in increased cellulose to amorphous form which is readily attacked by fungi. Dhillon *et al.* (1980) proposed similar reason for increase in SCP from delignified cellulose with sodium hydroxide. Iyayi (2004) reported that the highest percentage increase in protein was obtained with *A. niger* when wheat offal inoculated with the fungus.

Role of *T. harzianum* in decomposition of cellulose. On the basis of SCP production, the *T. harzianum* was selected for further study. From the data, it was evident that the maximum SCP (28%) was produced 5 days after incubation (Table 2). Furthermore, increase in incubation period did not enhance SCP production rather slight decrease in SCP production was observed. This might be due to autolysis of the fungal mycelium and similar to the previous results (Sekhon, 1975; Dhillon *et al.*, 1980). They ascribed similar reason of slight decrease in SCP after fifth day of incubation. Ofuya and Nwanjiuba (1990) have reported that fungal enzyme-controlled degradation responds to incubation time, pH and temperature of the medium. With fungal biomass increase, the nutrients in the substrate medium are quickly used up.

Table 3. Effect of different nitrogen sources on SCP production by *Trichoderma harzianum*

Nitrogen Sources	Dry weight (g)	Protein (%)	Protein mg/flask	Protein (mg/g) of the substrate
Ammonium nitrate	343	32.50	111	222
Ammonium chloride	310	27.50	85	170
Ammonium sulphate	356	23.90	85	170
Ammonium dihydrogen phosphate	344	30.00	103	206
Diammonium hydrogen phosphate	330	28.91	95	190
Potassium nitrate	390	32.90	128	256
Sodium nitrate	353	15.02	53	106
Urea	384	28.90	110	220

Effect of nitrogen sources on SCP production. Among eight different sources, the most suitable nitrogen source (potassium nitrate) was found to be the best for maximum SCP production, followed by ammonium nitrate and urea (Table 3). Other nitrogen sources showed poor SCP production. Similar results were reported by Dhillon and Chahal (1978).

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