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CELLULOSE DEGRADATION ACTIVITY BY VARIOUS MULTISPORE ISOLATES OF *PLEUROTUS* SPP. IN DIFFERENT SUBSTRATES

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Abstract

Mushrooms have achieved significant importance in many countries due to their high nutritive and genuine medicinal values as well as an income generative venture. Blessed with varied agro-climates, Indian weather is aptly suitable for the cultivation of edible mushrooms.The cellulose content was significantly more in sawdust (48.30 %) followed by groundnut haulm (41.72 %), sugarcane trash (35.68 %), paddy straw + saw dust (32.78 %). The lowest amount of cellulose was recorded in paddy straw (31.34 %) but which was on par with paddy straw + sugarcane trash containing 31.39 per cent. The isolate, *Pe x Po* reduced the cellulose content of paddy straw from 31.34 to 25.41 per cent which accounted for 18.92 per cent reduction in the cellulose content. Further, it was reduced the cellulose content of paddy straw + sugarcane trash by 11.61 per cent over standard parent. The lowest reduction in the cellulose content was observed with sawdust recording 6.23 per cent reduction over standard parent. Among the isolates, the least effective isolate, *Pf x Po* degraded cellulose content of paddy straw from 31.34 to 26.78 per cent.

***Key words*:** Cellulose, Pleurotus, Sugarcane trash and paddy straw.

Introduction

Mushrooms have achieved significant importance in many countries due to their high nutritive and genuine medicinal values as well as an income generative venture. Blessed with varied agro-climates, Indian weather is aptly suitable for the cultivation of edible mushrooms. The entire coastal belts of India running in to thousands of kilometers is a potent place to produce low cost speciality mushrooms which could supplement the protein deficiency and malnutrition, besides bringing in a sky - rocketing export market of a kind which is incomparable to any single cell protein (SCP) product (Kohlii, 2000).

It is estimated that about 355 million tonnes of crop residue is generated annually and about 170 million is left out posing problems for disposal (Tewari and Pandey, 2002). Even if one per cent of this agricultural waste is used to produce mushrooms, India will soon become a major mushroom producing country in the world. Mushroom production is the only biotechnological means available to convert these agricultural wastes into highly valuable edible proteins. So far around 5658 species of mushroom in 230 genera have been recorded from all over the world; where as from India 850 species spread over 115 genera have been reported. Of this 850 species about 20 are being commercially cultivated (Saini and Atri 1995).

Among these, the white button mushroom(*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), paddy straw mushroom (*Volvariella volvacea*) and milky mushroom (*Calocybeindica*) are popular among the commercial growers in India as the techniques for their cultivation have been well developed (Vijaya Khader *et al*., 1998). World mushroom production at present is estimated to be around 5 million tonnes/annum and is increasing @ 7 per cent/annum. The total mushroom production in India has increased from 4000 tonnes in 1955 to 30,000 tonnes in 1995 and it is estimated to be around 50,000 tonnes / annum (Tewari, 2004).

Agaricus bisporus is highly temperature specific, and its cultivation is restricted to temperate regions. But oyster mushrooms can be cultivated easily in tropical and subtropical regions. Hence, it is rightly named as "the crop of the future". *Pleurotus* spp. has the ability to degrade most of the lignocellulosic agro wastes, thus the cultivation of this mushroom is an efficient means for the conversion of agricultural wastes in to valuable edible proteins (Deepika Sud and Sharma, 2005).

The farmers and consumers have also developed preference towards *Pleurotus* spp. in recent years because of its advantages *viz.,*high nutritive value and easiness in cultivation using the farm wastes (Eswaran, 1998). Among the thirty eight species of *Pleurotus* existing in nature, only nine species are being cultivated under artificial condition (Jandaik, 1987). Every species has its own attributes and each is known for its yield, substrate utilization and wide temp. adoption (Ravichandran, 2001). Inspite of its easy cultivation methods and adaptation to wide range of temp., the production of *Pleurotus* spp. is very less when compared to button mushroom production in India. Hence, a need was felt for up scaling the yield potential of *Pleurotus* spp. for large scale production.

In general, enhancement in mushroom production has been made by agronomic practices rather than through genetic improvement of strains (Kapoor *et al.,* 1996). The possibility of strain improvement by means of mycelial anastomosis was also reported to be promising (Kneeborne *et al.,* 1972). Also the strain improvement through mycelial fusion between the

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multispore cultures of *Pleurotus* spp.was tried (Geetha, 1993) and the hybrids of the fusants were reported to yield more and bear larger fruiting bodies (Pandey and Tewari, 1994).

Materials and Methods

Organism

The pureculture of *Pleurotus* spp. (*Pleurotus citrinopileatus* (Fr.) Singer, *P. djamor* (Rumph.) Boedijn, *P. eous* (Berk) Sacc, *P. flabellatus* (Berk and Br.) Sacc., *P. florida* (Eger) and *P. ostreatus* (Jacq.Fr.) Kummer) were obtained from National Centre for Mushroom Research (NCMR) Chambaghat, Solan, Himachal Pradesh. The sub cultures were maintained on oat meal agar (OMA) medium.

Isolation and purification

The mushroom tissue was cut at the junction of the pileus and stipe using a sterile scalpel and surface sterilized with 95 per cent ethyl alcohol for one min. These bits were placed on OMA in sterile Petri dishes and incubated at room temp. (28±2° C) for seven days. The isolates were then purified by single hyphal tip method and maintained on OMA slants.

Preparation of spawn

Sorghum grain spawn was prepared by adopting the method described by Sivaprakasam (1980). Sorghum grains were partially cooked in water for 40 min. After draining the excess water, the grains were mixed with calcium carbonate at two per cent (w/w) to prevent adhesion of the grains and for optimizing pH. The grains were filled up to two-third volume of glass glucose drip bottles plugged with non-absorbent cotton wool, the mouths were wrapped and sterilized at a pressure of 15 psi. for two h. The grains were inoculated with pure cultures of the fungus and incubated at room temp. (28±2°C). All these were carried out under aseptic condition (Plate 1). The nature of the growth and time taken for complete colonization of the spawn were recorded.

Cultivation trials

Preparation of mushroom bed

Cultivation of *Pleurotus* was carried out in transparent polythene bags of 60 x 30 cm size and thickness of 100 gauges. Cylindrical beds were prepared using 0.5 kg of paddy straw on dry weight basis, following the method described by Eswaran 1998. The unchopped whole straw was made into coils and used. A layer of coiled paddy straw was placed at the bottom of polythene bag. Over this, a layer of spawn was placed. In this manner five layers of coiled paddy straw and four layers of spawn were placed in the polythene bag and then the bag was tied at the top. The mushroom beds were hung from the ceiling by means of ropes ("Uri" method) instead of the usual method of keeping them in tiers made of bamboo or casuarina stacks (Plate 2). Two holes were made in the polythene bags and the beds were kept in cropping room, where the temp. was maintained between 23 to 28° C and relative humidity between 80 to 90 per cent. Water was sprinkled regularly to maintain adequate moisture and relative humidity. The following yield parameters were studied in all the experiments.

Spawn run

Number of days taken for 100 per cent colonization/ mycelial coverage on the substrate was recorded as spawn run period.

Time taken for first harvest

The number of days required for first harvest of the sporophores from the date of spawning of the bed was recorded.

Weight of sporophores

The sporophores were weighted after harvest and yield per bed in g. was recorded.

Biological efficiency

The biological efficiency of *Pleurotus* spp. was calculated by

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of the mushrooms / bed}}{\text{Dry weight of the substrates / bed}} \times 100$$

Estimation of cellulose in substrate

Cellulose content of the substrates was estimated by adopting the method described by Updegraff, 1969. One hundred mg of the sample was mixed with 15 ml of nitric acid and acetic acid mixture (15 : 85 ml), boiled in a water bath for 20 min. and centrifuged at 4000 to 5000 rpm for 15 to 20 min. The residue was dissolved in 10 ml of 67 per cent sulphuric acid and diluted to 25 ml. To one ml of the diluted sample four ml of dist. water was added and kept in an ice bath to cool. To this 10 ml of cold anthrone reagent was added and the tubes were placed in a boiling water bath for 16 min. Later the tubes were cooled in an ice bath for two to three min. and kept at room temp. (28 ± 2°C) for 5 to 10 min. The OD values were measured in a spectrophotometer at 620 nm against a reagent blank.

Results

Cellulose degradation by various multispore isolates on different substrates

The cellulose content was significantly more in sawdust (48.30 %) followed by groundnut haulm (41.72 %), sugarcane trash (35.68 %), paddy straw + saw dust (32.78 %). The lowest amount of cellulose was recorded in paddy straw (31.34 %) but which was on par with paddy straw + sugarcane trash containing 31.39 per cent (Table 1). It is apparent from the data that the cellulose content of all the substrates tested was reduced by all the multispore isolates when compared to the standard parent. Among the multispore isolates, the maximum reduction in cellulose content was caused by Pe x Po in all the substrates tested. This was followed by Pc x Pe, Pc x Pfl, Pf x Po and standard parent (P. eous) in the decreasing order which differed significantly among themselves.

The isolate, Pe x Po reduced the cellulose content of paddy straw from 31.34 to 25.41 per cent which accounted for 18.92 per cent reduction in the cellulose content. Further, it was reduced the cellulose content of paddy straw + sugarcane trash by 11.61 per cent over standard parent. The lowest reduction in the cellulose content was observed with sawdust recording 6.23 per cent reduction over standard parent. Among the isolates, the least effective isolate, Pf x Po degraded cellulose content of paddy straw from 31.34 to 26.78 per cent; paddy straw + sugarcane trash from 31.39 per cent to 28.85 per cent and paddy straw + groundnut haulm from 32.42 to 29.97 per cent. The lowest reduction of cellulose from 48.30 to 46.92 per cent was observed with sawdust which worked out to only 2.85 per cent

Similarly, the standard parent (P. eous) reduced the cellulose content of paddy straw from 31.34 to 27.42 per cent which accounted for 12.50 per cent reduction followed by paddy straw + sugarcane trash where the cellulose content was reduced from 31.39 to 29.02 per cent (7.54 %).

Discussion

The oyster mushroom, Pleurotus spp effectively degrades lignin, cellulose and hemicellulose present in various substrates and converts these into nutritious food in the form of fruit body. The cellulose content of different substrates gradually decreased with the growth of the fungus. In the present study, it was found that cellulose degradation was more in paddy straw followed by paddy straw with sugarcane trash (1:1) combinations (Table 1).

Zadrazil (1978) reported the reduction in cellulose content in the substrate caused by P. florida. Similarly, P. sajor-caju reduced the cellulose content in the paddy straw from 48.3 to 27.9 per cent 32 days after incubation (Saxena and Rai, 1992a). Several earlier workers have reported that high cellulose content resulted in enhanced cellulase production which in turn was positively correlated with the yield of sporophores (Ramasamy and Kandasamy 1976; Sivaprakasam and Kandasamy 1981c).

Geetha and Sivaprakasam 1998b, found that P. djamor is an efficient degrader of cellulose than P. citrinopileatus in paddy straw substrate. P. djamor degraded the coirpith to the maximum level by decreasing the cellulose from 27.13 to 10.25 per cent (Ramamoorthy et al., 1999).The growth and fruiting of an individual mushroom fungus on a particular lignocellulosic substrate depend on its ability to utilize the major components depending on its ability to synthesize hydrolytic enzymes necessary to degrade the components to low molecular weight readily assimable compounds (Datta and Chakravarty, 2002).

Table 1: Cellulose degradation activity by various multispore isolates of *Pleurotus* spp. in different substrates

S.No	Substrates	0th day	*Pc x Pe*		*Pc x Pfl*		*Pe x Po*		*Pf x Po*		*P. eous*	
			CC	PR	CC	PR	CC	PR	CC	PR	CC	PR
1.	Paddy straw	31.34 (34.01)	25.45 (30.32)	18.79	25.77 (30.51)	17.77	25.41 (30.26)	18.92	26.78 (31.17)	14.55	27.42 (31.56)	12.50
2.	Sugarcane trash	35.68 (36.69)	33.14 (35.13)	7.11	33.26 (35.22)	6.78	32.88 (34.98)	7.83	33.47 (35.36)	6.19	33.56 (35.41)	5.94
3.	Saw dust	48.30 (44.02)	46.03 (42.70)	4.69	46.25 (42.86)	4.24	45.29 (42.30)	6.23	46.92 (43.22)	2.85	47.42 (43.51)	1.82
4.	Groundnut haulm	41.72 (40.23)	39.10 (38.70)	6.27	39.12 (38.70)	6.22	38.77 (38.51)	7.06	39.30 (38.82)	5.79	39.51 (38.94)	5.29
5.	Paddy straw + Sugarcane trash (1:1)	31.39 (34.07)	28.20 (32.07)	10.15	28.26 (32.12)	9.96	27.75 (31.82)	11.61	28.85 (32.48)	8.08	29.02 (32.58)	7.54
6.	Paddy straw + Saw dust (1:1)	32.78 (34.93)	30.09 (33.27)	8.18	30.26 (33.38)	7.67	29.96 (33.20)	8.60	30.48 (33.52)	7.00	30.61 (33.58)	6.59
7.	Paddy straw + Groundnut haulm (1:1)	32.42 (34.69)	29.42 (32.83)	9.24	29.82 (33.10)	7.99	29.40 (32.83)	9.32	29.97 (33.21)	7.53	29.99 (33.20)	7.47
SEd		0.54	0.28		0.21		0.27		0.22		0.28	
CD (P = 0.05)		1.09	0.56		0.42		0.56		0.44		0.55	

References

Deepika Sud and Sharma, B.M. (2005). Cultivation of some naturally occurring strains of oyster mushroom. *Mushroom Res*., **14(2)**: 60-62.

Eswaran, A. (1998). Studies on the physiological, cultural and post-harvest aspects of oyster mushroom Pleurotus eous (Berk.) Sacc. Ph.D. thesis, Annamalai Univ., India.

Geetha, D. (1993). Studies on oyster mushroom (Pleurotus spp.) Ph.D. Thesis, TNAU, Coimbatore p. 253.

Jandaik, C.L. (1987). Breeding potentials of Pleurotus species in India with special reference to Pleurotus sajor-caju. *Indian Mush. Sci.*, **2**: 355-360.

Kapoor, M., Sodhi, H.S. and Dhanda, S. (1996). Strategies for strain improvement in Pleurotus species. *Mushroom Res.*, **5**: 57-66.

Kneeborne, L.R., Schultz, P.G. and Patton, T.G. (1972). Strain selection and development by means of mycelial anastomosis. *Mush. Sci.*, **8**: 19-25.

Kohlii, M.S. (2000). Right technologies can help. Hindu survey of Indian Agriculture. pp : 199-200.

Pandey, M. and Tewari, R.P. (1994). Strategies for selection and breeding of edible mushrooms. ln: Advances in mushroom biotechnology (Eds.) Nair, N. C. Scientific publishers, Jodhpur. pp. 61-69.

Rangasamy, G., Kandasamy, T.K. and Ramasamy, K. (1975). Pleurotus sajor-caju (Fr.) Singer-a protein rich nitrogen fixing mushroom fungus. *Curr. Sci.*, **44**: 403-414.

Ravichandran, V. (2001). Studies on developing high yielding oyster mushroom with desirable qualities. Ph.D. Thesis, TNAU, Coimbatore, India, p. 180.

Saini, S.S. and Atri, N.S. (1995). Mshroom flora of Punjab In:

advances in Horticulture, 13: 374-386. Mushroom (Eds. K.L. Chadha and S.R. Sharma), Malhotra publishing House, New Delhi.

Sivaprakasam, K. and Kandasamy, T.K. (1981a). Effect of different types of spawn on sporophore production of Pleurotus sajor-caju (Fr.) Singer. *Madras Agric. J.* **68**: 178-182.

Tewari, R.P. and Pandey, N. (2002). Sizeable income generating venture. Hindu survey of Indian Agriculture. pp: 165-167.

Tewari, R.P. (2004). Now in the lime light. Hindu Survey of Indian Agriculture. pp: 132-133.

Updegraff, D.M. (1969). Utilization of cellulose from waste paper by Myrothecium verucaria. *Analy. Biochem*., **32**: 420-424.

Vijaya Khadar, Shobadevi, P., Sarojini, G., Alakanada, J. and Nayana Pandya (1998). Mushrooms for many uses. *Food Nut. News,* **3(1)**: 15-22.

Zadrazil, F. (1978). Cultivation of Pleurotus. In: The biology and cultivation of edible mushrooms. (Eds). Chang, S. T. and Hayes, W. A. Academic Press, New York. p. 521-557.