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LIGNIN 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. Among the various substrates, sawdust had the highest lignin content of 25.44 percent followed by groundnut haulm (16.92 %), sugarcane trash (12.03 %), paddy straw + sawdust (10.86 %) paddy straw + groundnut haulm (9.79 %) and paddy straw + sugarcane trash (9.22 %). The lowest lignin content (8.46 %) was observed in paddy straw. Degradation of lignin revealed that the isolate *Pe x Po*. recorded the maximum reduction of lignin in all the substartes when compared to other isolates and standard parent.

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).

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Among these, the white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), paddy straw mushroom (*Volvariella volvacea*) and milky mushroom (*Calocybe indica*) 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 percent/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.

Materials and Methods

Organism

The pure culture 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 × 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^{0}C and relative humidity between 80 to 90 percent. 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 percent 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

Biological efficiency (%) =

$$\frac{\textit{Fresh weight of the mushrooms / bed}}{\textit{Dry weight of the substrates / bed}}$$

Estimation of lignin in substrates

Lignin content of the samples was estimated gravimetrically following the method of Chesson (1978). One g of sample was added to five ml of conc. sulphuric acid (96 to 98%) and thoroughly mixed. It was transferred into a 500 ml conical flask containing 450 ml of dist. water and boiled for 10 min. The contents of the flask were filtered through glass filter. The acidic residues were washed to neutrality with dist. water. Lignin was expressed in terms of per cent on dry weight basis of the substrate.

Results

The results on the lignin degradation by different multispore isolates in the various substrates are presented

in table 1. Among the various substrates, sawdust had the highest lignin content of 25.44 percent followed by groundnut haulm (16.92%), sugarcane trash (12.03%), paddy straw + sawdust (10.86%) paddy straw + groundnut haulm (9.79%) and paddy straw + sugarcane trash (9.22%). The lowest lignin content (8.46%) was observed in paddy straw.

The lignin degradation was to the maximum extent (21.15% reduction) in paddy straw by *Pe x Po*, followed by in paddy straw + sugarcane trash (18.48% reduction). The lignin content in paddy straw + groundnut haulm was decreased from 9.79 to 8.01 percent accounting for 18.03 percent reduction over control. The least degradation (14.93%) of lignin was found to be in sawdust by *Pe x Po*.

The lignin degradation by *Pc x Pe* was the maximum (6.76%) in paddy straw which worked out to 20.05 percent reduction over control. The lignin content in paddy straw + sugarcane trash was reduced from 9.22 to 7.52 percent. The least degradation (13.46%) of lignin was found to be in sawdust by *Pc x Pe*.

The lignin content was reduced from 8.46 to 6.85 percent by *Pc x Pfl* in paddy straw which accounted for 19.00 percent reduction over standard parent which was followed by paddy straw + sugarcane trash from 9.22 to 7.52 percent (18.40% reduction) and paddy straw + groundnut haulm from 9.79 to 8.03 percent (17.96% reduction). The lowest lignin degradation by the isolate was observed in sawdust (13.32% reduction).

The isolate, *Pf x Po* was found to degrade the lignin content to the maximum extent in paddy straw substrate. The lignin content of this substrate was decreased from 8.46 to 6.86 percent which was 18.85 percent reduction over the control. It was followed by paddy straw + sugarcane trash, paddy straw + groundnut haulm, paddy straw + sawdust and sugarcane trash which recorded 18.35, 17.90, 17.21 and 17.10 percent reduction of lignin, respectively. The minimum degradation of lignin was observed in sawdust substrate accounting for 13.20 percent reduction over standard parent.

Lignin degradation by *P. eous* was the maximum in paddy straw with 6.87 percent which accounted for 18.75 percent reduction over control. The reduction of lignin with paddy straw + sugarcane trash was found to be reduced by 18.28 percent. Lignin in saw dust was reduced by *P. eous* from 25.44 to 22.13 percent, which was 13.00 percent reduction over the control.

Discussion

In the present investigation it was found that lignin content was more in sawdust (25.44%) and less in paddy straw (8.46%). The substrate with high lignin content had lesser yield of multispore isolates while that with low lignin content had higher yield (Table 1). This is in conformation with the report that sporophore production is positively correlated with cellulose content and negatively correlated with lignin concentration (Ansu Ouseph *et al.*, 2001). *Pleurotus sajor-caju* was a more efficient colonizer of the substrate because of more active hydrolytic enzyme system and its productivity in the substrate was much

Table 1: Lignin 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*	
			LC	PR	LC	PR	LC	PR	LC	PR	LC	PR
1.	Paddy straw	8.46 (16.95)	6.76(15.11)	20.05	6.85(15.16)	19.00	6.67(14.99)	21.15	6.86(15.19)	18.85	6.87(15.22)	18.75
2.	Sugarcane trash	12.03 (20.27)	9.96(18.41)	17.20	9.94(18.36)	17.16	10.31(18.74)	17.26	9.97(18.43)	17.10	9.98(18.43)	17.01
3.	Saw dust	25.44 (30.26)	22.01 (27.97)	13.46	22.05(28.00)	13.32	21.64(27.69)	14.93	22.08 (28.02)	13.20	22.13 (28.03)	13.00
4.	Groundnut haulm	16.92 (24.29)	14.09 (22.05)	16.70	14.10(22.05)	16.65	14.08 (22.05)	16.75	14.11 (22.05)	16.61	14.12 (22.07)	16.51
5.	Paddy straw + Sugarcane trash (1:1)	9.22 (17.65)	7.52(15.88)	18.43	7.52(15.88)	18.40	7.51(15.88)	18.48	7.53(15.91)	18.35	7.55(15.99)	18.28
6.	Paddy straw + Saw dust (1:1)	10.86 (19.27)	8.97(17.45)	17.37	8.98(17.45)	17.27	8.96(17.45)	17.43	8.99(17.42)	17.21	9.00(17.45)	17.15
7.	Paddy straw + Groundnut haulm (1:1)	9.79 (18.24)	8.02(16.42)	18.00	8.03(16.42)	17.96	8.01(16.42)	18.03	8.04(16.45)	17.90	8.06(16.53)	17.75
SEd		0.16	0.26	0.31		0.27		0.33		0.22		
CD (P=0.05)		0.33	0.53	0.63		0.53		0.67		0.45		

Figures in parentheses are arcsine transformed values, LC – Per cent lignin content, PR – Per cent reduction

higher. (Datta and Chakravarty, 2002).

In the present study, it was found that the substrates with high lignin content supported minimum growth of oyster mushroom. Lignin was found to affect adversely the activity and production of cellulases which might be the reason for poor growth and yield of mushroom in saw dust and other substrates rich in lignin, while the substrates with less lignin seems to favour cellulase activity there by increasing the growth and yield of mushrooms. The substrates with less lignin probably induced the more enzymatic activity resulting in higher yield of sporophores (Theradimani *et al*., 2002). Lignin degradation by *Pleurotus* spp was reported by several earlier workers (Platt *et al.,* 1984; Zadrazil, 1980; Hussain *et al.,* 1988).

Ouseph *et al.,* (2001) reported that *P.sajor-caju* is the most efficient lignin degrader followed by *P. platypus. P. ostreatus* was able to degrade upto 65 percent of the lignin present in cotton stalk within 21 days growth period (Platt *et al.,* 1983). Degradation of lignin in coirpith compost by *P.sajor-caju* has been reported by Ramasamy *et al*., (1985). Higher rate of lignin degradation in paddy straw was recorded by *P. citrinopileatus* (Geetha and Sivaprakasam, 1998b). All these reports corroborate and lend support to the present investigations.

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