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REGULAR ARTICLE

SEMI-SOLID FERMENTATION OF *PLEUROTUS OSTREATUS*

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ABSTRACT

Four strains of oyster mushrooms (*Pleurotus ostreatus*) KBB, M_1, S_1, T_1, were cultivated on lignocellulosis wastes such as wheat straw and cornstalks separately or in combination 1:1. The aim of this work was to choose the best substrate for individual strain of mushroom based on the growth characteristics and yield. Cultivation was realised in polyethylene bags and plastic containers at the temperature 22 ± 1 ^{o}C, low concentration of carbon dioxide, high air humidity 85 – 90% and sufficient lighting. Cultivation in polyethylene (PE) bags was at all strains more effective. There were achieved higher yields of fruiting bodies and lower time of colonization of substrate compared with cultivation in plastic containers.

Keywords: *Pleurotus ostreatus*, cultivation, substrates, colonization, yield, biological efficiency

INTRODUCTION

Cultivation of edible mushrooms is a biotechnological process for lignocellulosic organic waste recycling. Roughly 300 mushrooms species are edible, but only 30 have been domesticated and ten grown commercially **(Barny, 2009).** *Pleurotus* sp. is the second most

cultivated edible mushroom worldwide after *Agaricus bisporus*. To date approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently **(Rühl *et al.*, 2008).** These mushrooms have economical and ecological values and medicinal properties. They are able to colonize and degrade a large variety of lignocellulosic substrates and other wastes which are produced in agricultural, forest and food-processing industries **(Sánchez, 2010). Poppe (2000)** reported that there are about 200 kinds of waste in which edible mushrooms can be produced. However, mushroom production generates an enormous amount of used „spent" substrate which might also be a source of environmental contamination.

In general, mushrooms contain 90% water and 10% dry mater. Their nutritional value can be compared to those of eggs, milk and meat **(Oei, 2003).** *Pleurotus* species are rich source of proteins and an abundance of essential amino acids, minerals (Ca, P, K, Fe, Na) and also contain vitamins C, B-complex – thiamine, riboflavin, niacin and folic acid **(Kalač and Svoboda, 2000; Isiloglu *et al*., 2001; Sánchez, 2004; Çağlarırmak, 2007; Regula and Siwulski, 2007).** Oyster mushroom *Pleurotus ostreatus,* due to its documented probiotic properties and relatively high nutritive value, are recommended in numerous countries as an addition to the daily diet **(Wang *et al.,* 2000; Smith *et al.,* 2002; Florczak *et al.,* 2004; Bernaś *et al.,* 2006).**

P. ostreatus and other *Pleurotus* species are able to utilize ligninocellulosic waste materials for the production of industrially important lignolytic and cellulolytic enzymes **(Mikiashvili *et al.,* 2006).**

The most important step for successful cultivation of *Pleurotus* sp. is the preparation of lignocellulosic material to ward off saprophytic fungi like *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Monilia* sp., *Mucor* sp., *Rhizopus* sp., *Trichoderma* sp., which compete with the mushroom fungus during spawn run **(Yildiz *et al.,* 2002; Velazquez-Cedeno *et al.,* 2007).**

Used strain is of particular importance, since some workers develop an allergy that is identical to mushroom-workers lung and is associated with personal exposure to *Pleurotus* sp. spores. This problem has increased interest in the development of sporeless mutants for breeding sporeless strains **(Senti *et al.,* 2000; Obatake *et al.,* 2003).**

The aim of this work was to choose the best substrate for individual strain of mushroom based on the growth characteristics and yield.

MATERIAL AND METHODS

Preparation of production strains of *Pleurotus ostreatus*

Strains of *Pleurotus ostreatus* were obtained from the Department of Biochemistry and Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak Agricultural University, Nitra. The cultures KBB, M_1, S_1 and T_1 were obtained from spores from the pieces of the specific mushrooms and cultivation of pure cultures was carried out at 25 ^oC in biological thermostat during 10-14 days. The cultures were maintained on Petri dishes on 2% Potato-Dextrose agar (PDA) at 4 ^oC.

Spawn preparation

Spawn was prepared from wheat grains, which were cleaned and soaked in tap water approximately for 6 hours. Thereafter the excess water was removed and wheat grains were subsequently packed to polyethylene bags and sterilized in an autoclave at 121 ^oC for 20 min. to diminish contamination of the substrate. After sterilization, the bags were inoculated with actively growing mycelium of *Pleurotus ostreatus* from Petri dishes and incubated at 25 ^oC for 10-15 days, until the mycelium fully covered the grains.

Substrates for semi-solid fermentation of *Pleurotus ostreatus*

Wheat straw and cornstalks and their combination in 1:1 ratio as cultivation substrates were used. The substrates were chopped into a length of about 2 to 4 cm and filled into polyethylene bags. Afterwards the boiling water was added to the prepared substrates, the bags were tied up and pasteurization took about 5 hours. After reducing temperature the excess of water was drained off by cutting the bag corners. The preserved substrates were inoculated by the prepared spawn and put in the cultivation room.

Yield mushroom and biological efficiency

Total weight of all the fruiting bodies harvested from all three pickings were measured as total mushroom yield. The biological efficiency (BE – accumulated fresh weight of

mushrooms expressed on the basic of dry weight of initial substrate) was calculated by the formula given by **Ahmad-Khan *et al.* (2012).**

Semi-solid fermentation of *Pleurotus ostreatus*

The first stage involved the production of pure mycelium of the specific mushroom strain. The pasteurized substrates was spawned and filled into clear polyethylene bags (size 50 x 30 cm) or into plastic containers (dimension 50 x 40 x 12 cm), which were covered by plastic wrap. Cultivation of spawned substrates was caried out in a room at the temperature of 15-17 ^{o}C and relative humidity 80-85%. Then the bags and containers were maintained under optimal conditions that are favorable for fruiting bodies, i.e. enough of light, temperature 22 ± 1 ^{o}C, humidity in the mushroom room 85-90%, airflow and low carbon dioxide content was secured. The mushrooms have begun to form around the edges of bag perforations. The mushrooms were harvested from the substrate approximately 4 to 5 weeks after spawning depending on strain, amount of supplement used and temperature of spawn run.

RESULTS AND DISCUSSION

In this work we tried to find out suitability of agricultural wastes as substrates for semi-solid fermentation of *Pleurotus ostreatus* by different ways of cultivation for different strains of *Pleurotus ostreatus* based on grown characteristics and adjusted yields. For successful mushroom production the choice of the strain used in the cultivation is crucial. A strain with a high ability to invade the substrate and to fruit, diminishes time of incubation and enhances productivity. The shortest time of cultivation of substrate was 21 days. It was monitored on wheat straw in polyehtylene bags on strains T_1 and M_1. It corresponds to results of **Ficior *et al.* (2010),** who investigated the influence of strain on the rate of intergrowth substrate. He used three cellulose substrates, corn-cobs, wheat straw and beechen sawdust in different ratio and the time of colonization was 21 days on straw and on substrate, which contained the straw in big ratio.

In our conditions the longest intergrowth of the cornstalk with the strain M_1 lasted 30 days in plastic containers. **Hasan *et al.* (2010)** recorded formation of the first fruiting bodies of *Pleurotus ostreatus* from 3 to 8 days after putting the substrate in the cultivation room. We recorded some differences at our strains of *Pleurotus ostreatus* related to formation of first fruiting bodies. The strains S_1 and KBB have begun to form the fruiting bodies an average 31

± 1 days after spawning and strains T_1 and M_1 about 34 ± 1 days after spawning of the substates. From the point of view of growth mycelium (22 days) and formation of first fruiting bodies (31 days) the most suitable substrate was the wheat straw. Slowlier growing mycelium was recorded on cornstalk (24 and 34 days) (Tab 1). Our cultivation conditions (enough of light, temperature 22 ± 1 ^{o}C, humidity in the mushroom room 85-90%, ventilation and low contents of CO_2) had good influence on growth of *P. ostreatus* in bags, but not in plastic containers. The strain S_1 on wheat straw and cornstalk 1:1 and strain M_1 on cornstalk didn't create the fruiting bodies. **Sánchez *et al.* (2002)** also recorded, that *P. ostreatus* can breed on different agricultural wastes, but yield depends on productive strain and on physical character of the substrate.

Table 1 Growth characteristics of *Pleurotus ostreatus*

Substrate	Way of cultivation	Strain/Days							
		KBB		M_1		S_1		T_1	
		IS	FFB	IS	FFB	IS	FFB	IS	FFB
Wheat straw	Polyethylene bags	22	31	21	32	22	31	21	32
	Plastic containers	24	31	22	32	24	31	22	32
Cornstalks	Polyethylene bags	22	29	24	39	22	29	22	39
	Plastic containers	27	35	30	-	27	35	22	35
Wheat straw + cornstalks 1:1	Polyethylene bags	24	31	22	35	24	31	22	35
	Plastic containers	-	-	22	32	27	-	22	32
		Ø 23	**Ø 31**	**Ø 23**	**Ø 34**	**Ø 24**	**Ø 31**	**Ø 22**	**Ø 34**

Legend: IS – intergrowth of substrate, FFB – first fruiting bodies

The biggest yield of fresh mushroom was obtained by strain S_1 (1,083.48 g) on the cornstalks in the polyethylene (PE) bags with biological efficiency (BE) 135.43% and total yield fruiting bodies of this strain on wheat straw in PE bags was 985.62 g with BE 123.20%. During cultivation of other strains significantly lower yields were recorded. It is interesting, that the lowest total yield was registered at the same strain (S_1) as the biggest one. On wheat straw 15.23 g fruiting bodies with BE 1.90% by cultivation of its strain was obtained (Tab 2).

Table 2 Yields of *Pleurotus ostreatus*

Substrate	Way of cultivation	Strain							
		KBB		M_1		S_1		T_1	
		TY	BE	TY	BE	TY	BE	TY	BE
Wheat straw	PE bags	220.19	27.52	228.00	28.50	985.62	123.20	205.10	25.63
	Plastic containers	463.11	57.89	113.80	14.22	15.23	1.90	236.11	29.51
Cornstalks	PE bags	747.57	93.44	90.53	11.31	1,083.48	135.43	305.47	38.18
	Plastic containers	523.43	65.43	0.00	0.00	307.27	38.41	219.74	27.47
Wheat straw + cornstalks 1:1	PE bags	736.86	92.10	284.46	35.56	492.37	61.55	453.68	56.71
	Plastic containers	0.00	0.00	81.50	10.19	0.00	0.00	219.74	27.47

Legend: TY – total yield of *P. ostreatus* (g), BE – biological efficiency (%)

The crop of oyster mushrooms was harvested in three flushes. The maximum yield was obtained in the first flush, the second and the third flush yield was decreasing. Maximum yield from the three flushes was 556.8 g recorded on cornstalks in PE bags and 262.6 g in plastic containers, following by wheat straw with yield 409.7 g in PE bags and 207.1 g in containers. Substrate from wheat straw and cornstalks gave the lowest average total yield and also biological efficiency (Tab 3).

Table **3** Influence of substrate on total yield of four investigate strains

Substrate	Way of cultivation	Average values in three flushes		Arithmetic mean with standard deviation and variation coefficient		
		TY	BE	TY (bags + containers)	VC	BE (bags + containers)
Wheat straw	Polyethylene bags	409.7	51.2	**308.40 ± 143.30**	**46.47**	**38.5 ± 17.91**
	Plastic containers	207.1	25.9			
Cornstalks	Polyethylene bags	556.8	69.6	**409.70 ± 207.99**	**50.77**	**51.2 ± 25.99**
	Plastic containers	262.6	32.8			
Wheat straw + cornstalks 1:1	Polyethylene bags	491.8	61.5	**296.10 ± 276.78**	**93.48**	**37.0 ± 34.60**
	Plastic containers	100.4	12.6			

Legend: TY – total yield of *P. ostreatus* (g), VC – variation coefficient, BE – biological efficiency (%)

CONCLUSION

Pleurotus ostreatus, also known as „oyster mushroom“ or „hiratake“, requires shorter growth time in comparison with other edible mushrooms. Also, the substrates used for cultivation do not require sterilization, only pasteurization which is less expensive. Fruiting bodies of this mushroom are not often attacked by diseases and pets and they can be cultivated in a simple and cheap way. A high percentage of the substrate is converted to fruiting bodies making *Pleurotus ostreatus* an excellent choice for mushroom cultivation.

Cultivation in polyethylene (PE) bags was at all strains more effective, were achieved higher yields of fruiting bodies and lower time of colonization of substrate compared with cultivation in plastic containers.

The cornstalks are recommended as the best substrate for the cultivation of our strains of *Pleurotus ostreatus*.

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