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Some Physiological Aspects of the Cultivation of *Lentinula edodes* (Berk.) Singer

Nina A. Bis'ko, Victor T. Bilay
Department of Mycology, N.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 2. Tereshchenkivska Str., 252601, Kiev, Ukraine
Fax: (944) 224 61 71

ABSTRACT: The mycelial growth of 19 strains of *Lentinula edodes* was investigated on different nutrient media, plant substrates and on their mixtures. The results revealed substantial phenotypic variability between the strains of *L. edodes* as to their growth on nutrient media and plant substrates. The rate of mushroom mycelial growth was higher on oak sawdust and buckwheat husk than on flax boon and wheat straw. It was determined that the addition of oak sawdust to sunflower husks, wheat straw or flax boon had a positive influence on the rate of growth of all strains. A mixture of oak sawdust with wheat bran (4:1) was the most favorable for fruiting of all strains of *L. edodes* strains by 17 to 54% while lignin decreased 1 to 15%. Using, SEM it was shown that there are different stages of *L. edodes* mycelial growth on substrates.

1 INTRODUCTION

The main components for the preparation of traditional substrates for cultivation of *Lentinula edodes* (Berk.) Sing. in Asian countries are the mixture of oak sawdust with rice bran. Shiitake farmers in some countries use substrates that are prepared from sawdust of different trees, wheat straw, waste after processing coffee, cacao, citrus and other components (Przybylowicz and Donoghue 1990, Siwulski and Gapinski 1991, Ruiz *et al.* 1994). During our experiments we studied physiological aspects of the *L. edodes* strains growth on different nutrient media and plant substrates which are important for the industrial cultivation of this mushroom in Ukraine.

2 MATERIALS AND METHODS

2.1 *Strains*

In our experiments we used 19 strains of *Lentinula edodes* (Berk.) Sing. from the collection of the Department of Mycology of the N.G. Kholodny Institute of Botany (Kiev, Ukraine). These strains were taken from collections of several Institutes in Japan, Czech Republic, Netherlands, North Korea and Poland. Pure cultures of *L. edodes* were maintained on beer wort agar medium.

2.2 *Media and substrates*

Growth rates of mycelium and morphology the colony of different strains of *L. edodes* were studied at 28°C on nutrient media — oat water extract agar medium (w.c.a.m) — mixture of onion with haricot bean w.e.a.m. (1:1.), mixture of onion with potato w.e.a.m. (1:1), mixture of potato with carrot w.e.a.m. (1:1), maize w.e.a.m. (Semcrdzieva 1966), grape refuse w.e.a.m., oak sawdust w.e.a.m., beer wort agar, and substrates — wheat straw, buckwheat husks, flax boon, mixture of wheat straw with oak sawdust (1:1), mixture of buck wheat husk with oak sawdust (1:1), mixture of sunflower husk with oak sawdust (1:1), mixture of flax boon with oak sawdust (1:1), oak sawdust, grape refuse, grape refuse after the extraction with cellulases and pectinases. The substrates were watered to obtain a moisture content of 65 to 70% and sterilized for 1 hr at 121°C.

For our studies of fructification of *L. edodes* we used the following substrates — mixtures of oak sawdust or alder sawdust or pine sawdust with brewing waste in a weight ratio of 10:1 and a mixture of oak sawdust with wheat bran (4:1). After watering, the moisture contents of substrates were 65 to 70%. Moistened substrate (2 kg) was placed in polypropylene bags and sterilized 1 hr at 121°C.

2.3 *Conditions for cultivation*

After cooling, bags with substrates were inoculated with wheat grain spawn at a 4% ratio of substrate weight. For mycelial growth the bags were incubated in a dark room at 26 to 28°C. Fructification of *L. edodes* occurred under room conditions of air temperature at 15 to 17°C, humidity of 80 to 90%, and light 150 lux.

2.4 *Chemical analysis*

Changes in grape refuse composition after mycelial growth of *L. edodes* was examined. The content of cellulose (Pleshkov 1976), lignin (Obolcnskaja *et al.* 1955) and total nitrogen according to Kjeldahl's method (Pleshkow 1976), was determined. Crude protein contents were calculated as total nitrogen x 6.25.

2.5 *Scanning electron microscopy*

The mycelial growth of *L. edodes* strains on the oak sawdust and grape refuse was studied by the scanning electron microscopy (SEM). Samples of each substrate with mycelium of *L. edodes* were prepared by vapor fixing (2% OsO_4 for 48 hr), coated with gold and examined using a JEOL JSM 35-C Scanning Electron Microscope.

3 RESULTS AND DISCUSSION

Analysis of data given in Fig. 1 showed that *L. edodes* grew on all tested media. The growth of the mycelium of strain 368 was the fastest on the mixture of onion with potato w.e.a.m., oak saw dust w.e.a.m. and grape refuse w.e.a.m. The mycelium of *L. edodes* strains 351, 352 and 362 grew faster on beer wort agar medium than other strains of this mushroom (Fig. 1). Results of our experiments show that complete overgrowth of oak w.e.a.m., a mixture of potato with carrot w.e.a.m., and maize w.e.a.m. occurred on the 13th day. Oak sawdust w.e.a.m., grape refuse w.e.a.m., mixture of onion with potato w.e.a.m. were overgrown by the 12th day. Beer wort agar was overgrown by the 10th day.

Lentinula edodes grew most slowly on wheat straw in comparison to other substrates (Fig. 2). The fastest rate of mycelial growth of *L. edodes* strains was observed on oak sawdust, buckwheat husks, grape refuse after the extraction with enzymes. Strain 368 was different from the others by its higher rate of growth on all tested substrates. We should note the great variability of growth rate of *L. edodes* strains on different substrates. For example, strain 351 grow more slowly than others on all substrates except a mixture of buckwheat husk with oak sawdust and grape refuse (Fig. 2).

It was found that addition of oak sawdust to plant substrates in weight ratio 1:1 has a positive influence on the growth rate of the mycelium of *L. edodes* strains, except substrates from mixture of oak sawdust

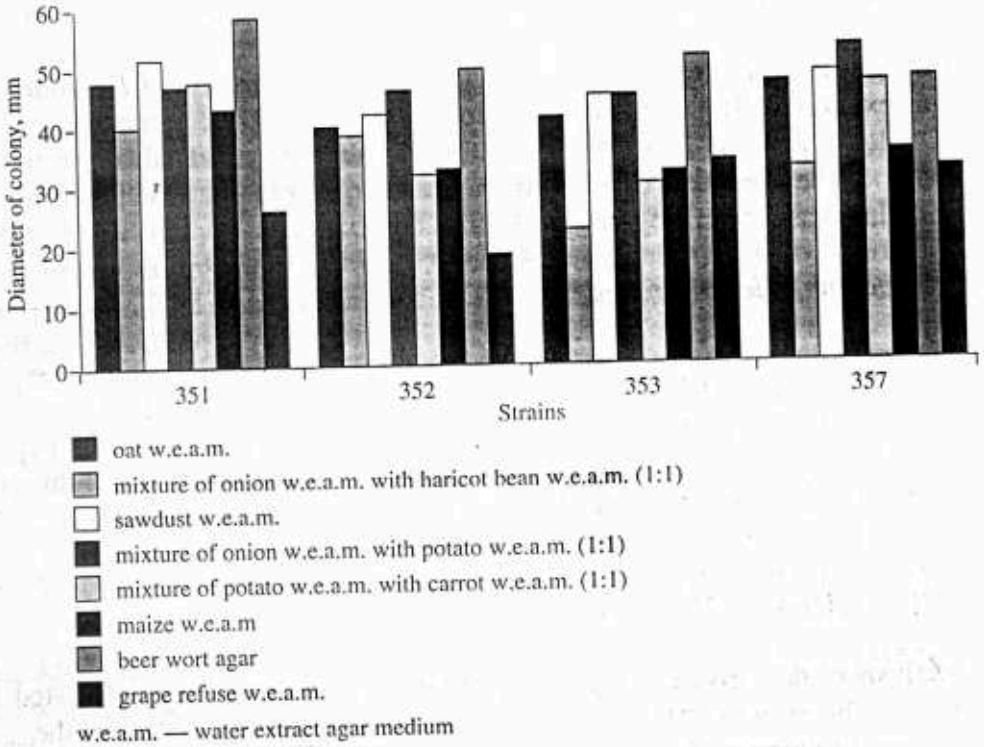

Fig. 1. Growth of *L. edodes* strains on agar media of different compositions (7 days).

with buckwheat husks (Fig. 2). The most favorable effect on the growth of mushroom mycelium was observed on the substrate containing a mixture of oak sawdust with wheat straw. Thus, the diameter of the colony of strains 368, 352, 355 on this substrate was increased by 68 to 77%, the diameter of the colony of strain 351 by 46% in comparison with those on wheat straw. The diameter of colony of the studied strains on the mixture of flax boon with oak sawdust was increased by 14 to 36%, on the mixture of sunflower husk with oak sawdust by 10 to 59% in comparison with these substrates without addition of oak sawdust (Fig. 2).

The highest yield of *L. edodes* strains (50 to 60% of the dry weight of substrate) was obtained on the mixture of oak sawdust with wheat bran (4:1). The yield of studied strains of *L. edodes* on the substrates from oak sawdust with brewing waste (10:1) was 20 to 25% of the dry weight of substrates, on the mixture of the alder sawdust with brewing waste (10:1) was 20 to 25% of the dry weight of substrates, on the mixture of the alder sawdust with brewing waste (10:1) 28 to 30%, on the

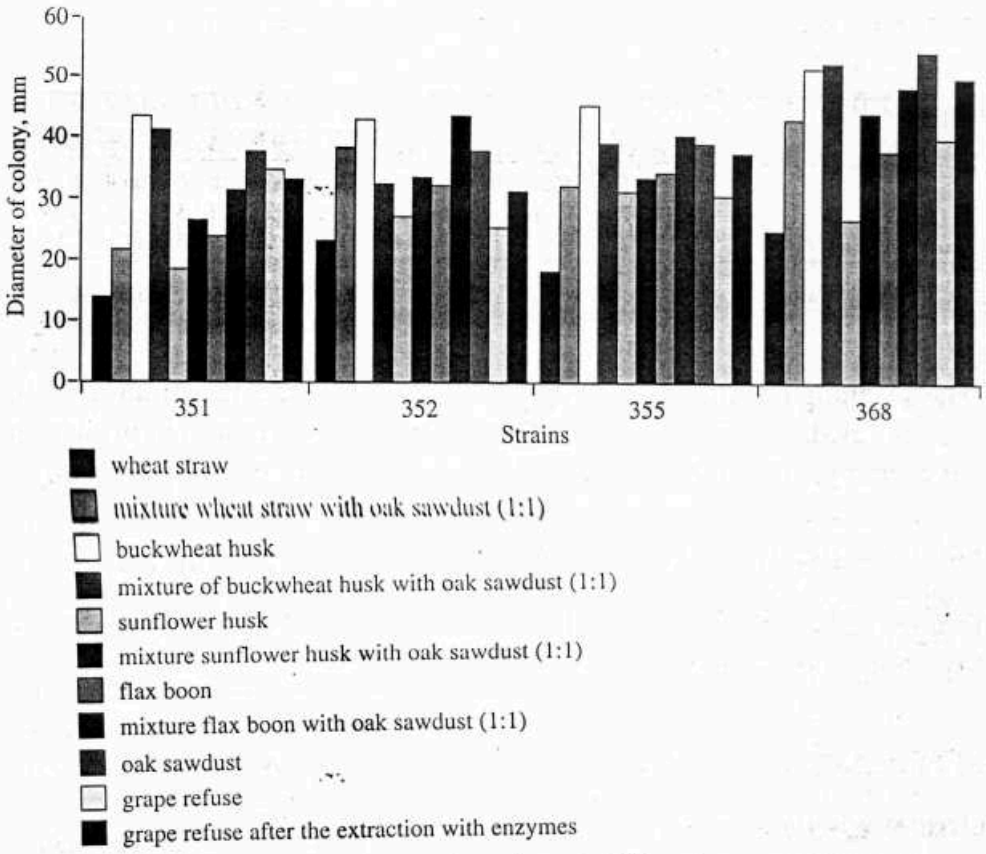

Fig. 2. Growth of *L. edodes* strains on plant substrates (7 days).

mixture of the pine sawdust with brewing waste (10:1) 19 to 20%. Considerable decrease of the content of cellulose in the grape refuse after the growth of *L. edodes* mycelium (by 17 to 54%) and lignin (by 1 to 15%) in comparison with uninoculated substrate was noted (Table 1). There was substantial phenotypic variability between the strains as to the grape refuse. For example, after the cultivation of *L. edodes* strain 359 on this substrates the content of lignin was decreased by 1%, but after the cultivation of strain 353 on the same substrates the content of lignin was decreased by 15.9%. During the growth of *L. edodes* mycelium on different substrates the content of crude protein was increased (Table 1). The quantity of the crude protein in grate refuse was 4.7 to 7.6 times higher than in uninoculated substrate depending on the different metabolic activity of the *L. edodes* strains. The same regularity was noted during mycelial growth of *Pleurotus ostreatus* strains on different substrates (Bis'ko *et al.* 1986).

Table 1. Changes in substrate composition after mycelial growth of *Lentinula edodes* strains on grape refuse.

Indices (% a.d.m.)	Strains									
	351	353	355	359	360	361	363	365	368	Control*
Lignin	64.2	56.8	59.7	66.9	63.6	63.4	65.6	63.6	65.4	67.6
Cellulose	8.4	7.1	7.8	11.5	10.4	11.2	12.9	12.3	8.5	15.7
Crude protein (Nx6.25)	12.5	14.2	12.8	10.3	9.8	11.4	9.1	10.6	14.6	1.9

*Uninoculated

The scanning electron microscopy studies of *L. edodes* mycelium growth on oak sawdust and grape refuse showed certain differences in mushroom mycelium growth. On oak sawdust *L. edodes* did not grow significantly on the substrate surface and on the 5th to 10th days formed aerial mycelium (photographs not available). At the same time on grape refuse a significant growth of *L. edodes* mycelium was observed on the substrate surface only after 10 to 15 days where a considerable amount of aerial mycelium was formed.

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