BIOTECHNOLOGY OF VINEYARD AND WINERY WASTES RECYCLING THROUGH *IN VITRO* CULTURES OF SOME EDIBLE MUSHROOM SPECIES

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Abstract
Every year, in Romania huge amounts of winery and vineyard wastes cause serious environmental damages in vineyards as well as nearby winery factories, for instance, by their burning on the soil surface or their incorporation inside soil matrix. In this respect, the main aim of this work was to establish the best biotechnology of winery and vineyard wastes recycling by using them as appropriate growth substrata for edible mushrooms. According to this purpose, two mushroom species of Basidiomycetes group, namely Lentinula edodes and Pleurotus ostreatus were used as pure mushroom cultures in experiments. All mycelia mushroom cultures were incubated for 120–168 h. During the incubation time period, all the spawn cultures were maintained in special growing rooms, designed for optimal incubation at 23°C. In the next stage of experiments, the culture composts for mushroom growing were prepared from the lignocellulose wastes as vine cuttings and marc of grapes in order to be used as substrata in mycelia development and fruit body formation. All the physical and chemical parameters that could influence the mycelia growing as well as fruit body formation of L. edodes and P. ostreatus were compared to the same fungal cultures that were grown on poplar logs used as control samples.

Keywords: biotechnology, biomass, edible mushrooms, recycling, vineyard and winery wastes

1. INTRODUCTION

The agricultural works as well as the industrial activities related to vine crops and wine processing have generally been matched by a huge formation of wide range of waste products. Many of these lignocellulosic wastes cause serious environmental pollution effects, if they are allowed to accumulate in the vineyards or much worse to be burned on the soil (Beguin and Aubert, 1994; Carlile and Watkinson, 1996). The solid substrate fermentation of plant wastes from agro-food industry is one of the challenging and technically demanding of all biotechnologies known to humankind (Chahal and Hachey, 1990; Chahal, 1994). The major group of fungi to degrade cellulose and lignocellulose are the edible mushrooms of Basidiomycetes Class (Leahy and Colwell, 1990; Moser, 1994). The main aim of this work was to find out the best biotechnology of recycling the winery and vineyard wastes by using them as a growing source for edible mushrooms and, last but not least, to protect the vineyard ecosystems (Petre, 2002; Petre et al., 2007). Taking into consideration that most of the edible mushrooms species requires a specific micro-environment including complex nutrients, the influence of all physical and chemical factors upon fungal biomass production and mushroom fruit bodies formation has been studied by testing new biotechnological procedures (Petre et al., 2008).

2. MATERIALS AND METHODS

According to the main purposes of this work, two fungal species of Basidiomycetes group, namely *Lentinula edodes* (Berkeley) Pegler (folk name: Shiitake) as well as *Pleurotus ostreatus* (Jacquin ex Fries) Kummer (folk name: Oyster Mushroom) were used as pure mushroom cultures isolated by authors from the natural environment and now being preserved in the local collection of the University of Pitesti. The stock cultures were maintained on malt-extract agar (MEA) slants (20% malt extract, 2% yeast extract, 20% agar-agar). Slants were incubated at 25°C for 120-168 h and stored at 4°C. The pure mushroom cultures were expanded by growing in 250-ml flasks containing 100 ml of liquid malt-extract medium at 23°C on rotary shaker incubators at 110 rev min^{-1} for 72-

120 h. To prepare the inoculum for the spawn cultures of *L. edodes* and *P. ostreatus* the pure mushroom cultures were inoculated into 100 ml of liquid malt-yeast extract culture medium with 3-5% (v/v) and then maintained at 23-25°C in 250 ml rotary shake flasks.
The experiments of inoculum preparation were set up under the following conditions: constant temperature, 25°C; agitation speed, 90-120 rev min^{-1}; initial pH, 5.5–6.5. All the seed mushroom cultures were incubated for 120–168 h. After that, the seed cultures of these mushroom species were inoculated in liquid culture media (20% malt extract, 10% wheat bran, 3% yeast extract, 1% peptone) at pH 6.5 previously distributed into rotary shake flasks of 1,000 ml. During the incubation time period, all the spawn cultures were maintained in special culture rooms, designed for optimal incubation at 25°C. There were prepared three variants of culture compost made of marc grapes and vineyard cuttings in the following ratios: 1:1, 1:2, 1:4 (w/w).
The vineyard and winery wastes were mechanically pre-treated by using an electric grinding device to breakdown the lignin and cellulose structures in order to make them more susceptible to the enzyme actions (Ropars et al., 1992; Verstraete and Top, 1992; Wainwright, 1992). All the culture compost variants made of ground vineyard and winery wastes were transferred into 1,000 ml glass jars and disinfected by steam sterilization at 120°C for 60 min. When the jars filled with composts were chilled they were inoculated with the liquid spawn already prepared. Each culture compost variant for mushroom growing was inoculated using liquid spawn having the age of 72–220 h and the volume size ranging between 3–9% (v/w). During the period of time of 18–20 d after this inoculation, all the mushroom cultures had developed a significant mycelia biomass on the culture substrata made of vineyard cuttings and marc of grapes (Smith, 1993; Stamets, 2008).

3. RESULTS AND DISCUSSION

The effects induced by some additional ingredients as carbon sources upon the mycelia growing during the incubation were investigated. As it can be noticed in figure 1, each carbon source was added to the basal composts at a concentration level of 5% (w/w) and the incubation time period lasted for 168-288 h.

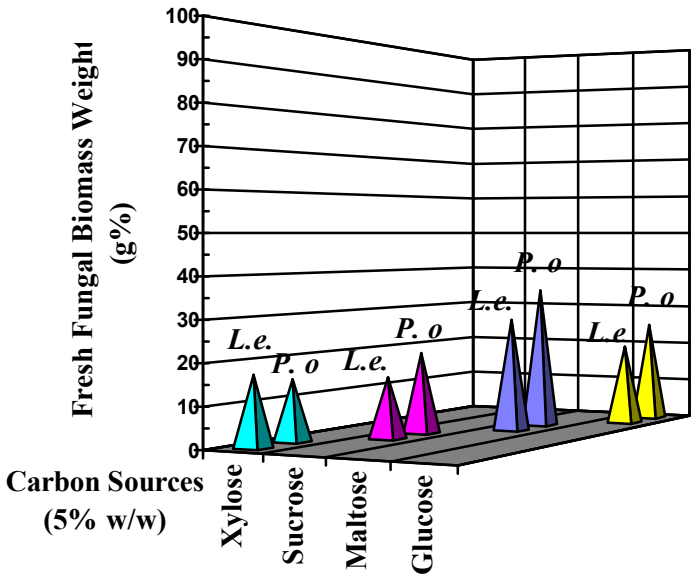

Figure 1. Comparative effects of carbon sources upon mycelia growing of p. Ostreatus (p.o.) And l. Edodes (l.e.)

Maltose, as one of all tested carbon sources, had shown the highest influence upon the mycelia growing and fresh fungal biomass production about of 28–35g%. The effects of nitrogen sources were registered as they are shown in figure 2.

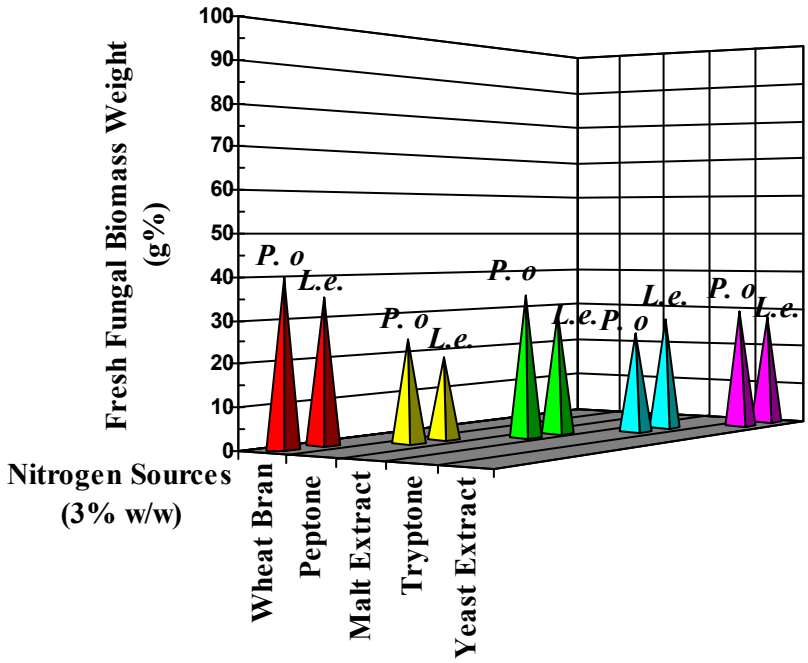

Figure 2. Comparative effects of nitrogen sources upon mycelia growing of p. Ostreatus (p.o.) And l. Eodes (l.e.)

Among the various mineral sources examined $CaCO_3$ yielded the best mycelia growing as well as fungal biomass production at 28-32 g% and for this reason it was registered as the most appropriate mineral source (Fig. 3).

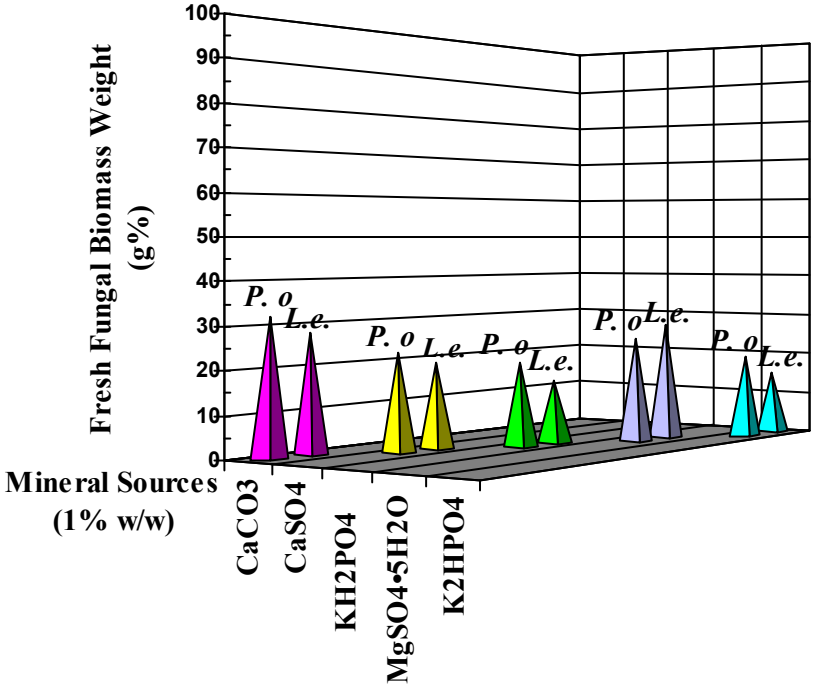

Figure 3. Comparative effects of mineral sources upon mycelia growing of p. Ostreatus (p. O.) And l. Edodes (l.e.)

From the tested nitrogen sources, wheat bran was the most efficient upon the mycelia growing and fungal biomass producing at 35-40 g% fresh fungal biomass weight, being closely followed by the malt extract at 25–30 g%. Peptone, tryptone and yeast extract are also well known nitrogen sources

for fungal biomass synthesis but their efficiency in these experiments was relatively lower than the mycelia growing and fungal biomass production induced by the wheat bran added as natural organic nitrogen sources (Smith, 1993; Stamets, 2008).
According to the registered results of the performed experiments the optimal laboratory-scale biotechnology for edible mushroom cultivation on composts made of marc of grapes and vineyard cuttings was established (Fig. 4).

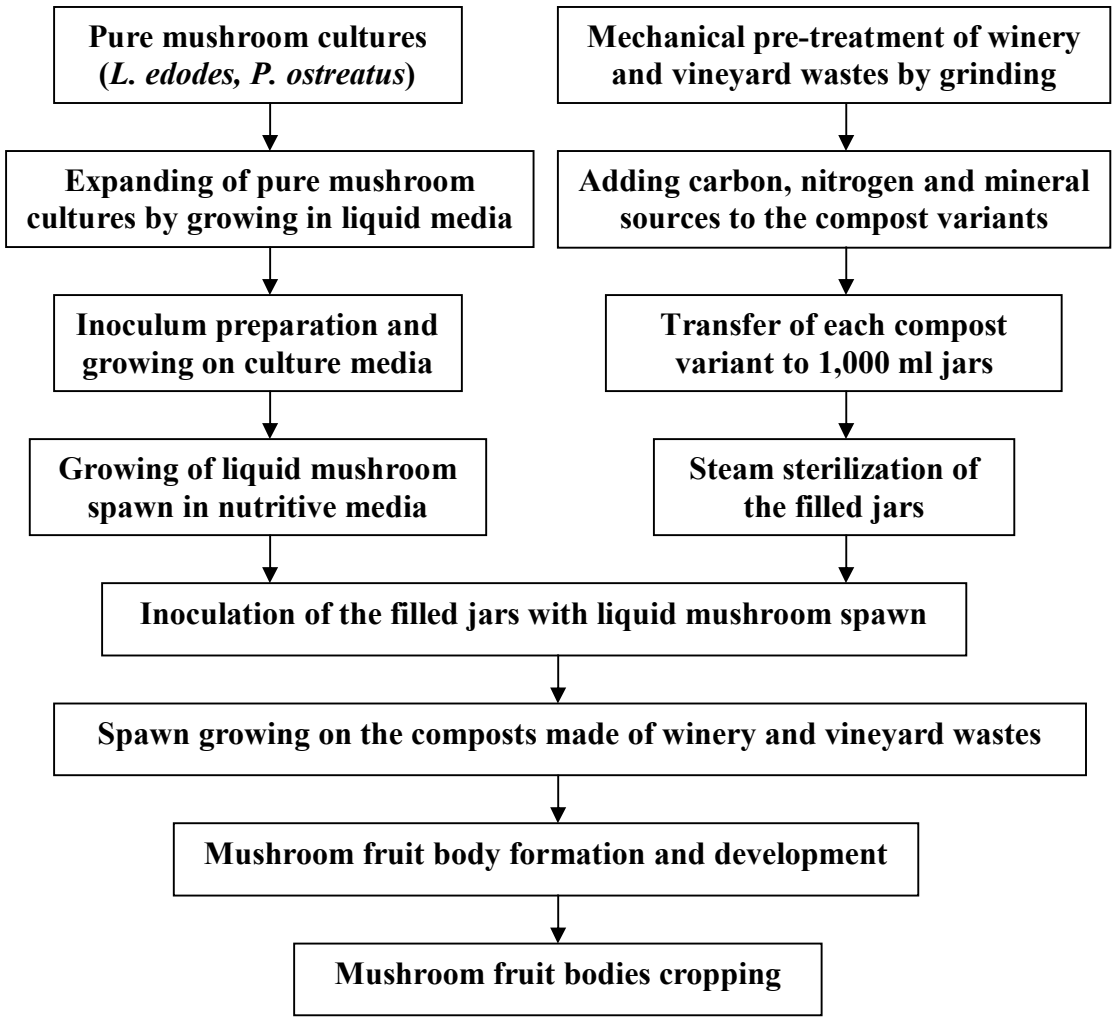

Figure 4. Scheme of laboratory-scale biotechnology for edible mushroom production by recycling winery and vineyard wastes

All the experiments were carried out for 288 h at 25°C with the initial pH 6.5 and all data are the means of triple determinations carried out on the variants of composts made of vineyard cuttings and marc of grapes in the ratio 1:4. Similar experiments concerning such techniques of mushroom cultivation were made by Stamets as well as other researchers, but the culture substrata were different (Smith, 1993; Stamets, 2008). Also, other mineral sources, such as $MgSO_4 \cdot 5\ H_2O$ have also shown a good influence upon the fungal biomass growing (Verstraete and Top, 1992; Wainwright, 1992). The mineral sources K_2HPO_4 and KH_2PO_4 as essential phosphates could improve the pH level through their buffering action, but they were less appropriate for mycelia growing in submerged as well as in surface cultures of mushrooms.
The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30–60 d, depending on each fungal species used in experiments. During the whole period of fruit body formation, the culture parameters were set up and maintained at the following levels,

depending on each mushroom species: air temperature, 15–17^{o}C; the air flow volume, 5–6m^{3}/h; air flow speed, 0.2–0.3 m/s; the relative moisture content, 80–85%, light intensity, 500–1,000 luces for 8–10 h/d. The final fruit body production of these mushroom species used in experiments was registered between 1.5 – 2.8 kg relative to 10 kg of composts made of vineyard and winery wastes.

4. CONCLUSIONS

1. The registered data revealed that by applying this biotechnology, the winery and vineyard wastes could be recycled as useful raw materials for culture compost preparation in order to get edible mushrooms.
2. Maltose, as one of all tested carbon sources, has shown the highest influence upon the mycelia growing and fresh fungal biomass production about of 28–35g%.
3. Among the five nitrogen sources examined, wheat bran was the most efficient upon the mycelia growing and fungal biomass production of *L. edodes* and *P. ostreatus*, at 35-40 g% fresh fungal biomass weight, closely followed by malt extract at 25–30 g%.
4. $CaCO_3$ yielded the best mycelia growing as well as fungal biomass production at 28-32g% and was registered as the best mineral source.
5.. The final fruit body productions of these two mushroom species were registered between 1.5–2.8 kg relative to 10 kg of composts made of vineyard and winery wastes.

5. ACKNOWLEDGEMENTS

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