

SHORT COMMUNICATION

Chemical and productivity characterization of parental and hybrid strains of *Lentinula edodes* cultivated in different agricultural residues

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ABSTRACT

Productivity of parental and hybrid strains of *Lentinula edodes* cultivated with two mixtures of agricultural wastes (mixture 1: peat moss, banana leaves, oak sawdust, millet seed, cotton seed hull and CaCO₃, and mixture 2: wheat straw, oak sawdust, cotton seed hull and CaCO₃), the chemical composition of the fruit bodies and also the antioxidant activities of hexanic extracts of the mushrooms were evaluated. The strains of *Lentinula edodes* (LEN97 and LEN95₄XLEC24₁) cultivated on both mixtures produced fruit bodies with highest protein contents since 16.71 to 17.10%. The hybrid strain of *Lentinula edodes* strain (LEN95₄XLEC24₁) cultivated on both mixtures showed the highest biological efficiencies since 74.61 to 82.80% and production rates between 1.21 and 1.30%, and also their hexanic extracts presented the highest antioxidant effects ranged from 8.01 to 9.84 mg mL⁻¹. The use of hybrid strain of *Lentinula edodes* can be used to improve the production of this genera.

Keywords: Agricultural wastes; antioxidant activity; chemical composition; *Lentinula edodes*; productivity.

INTRODUCTION

Lentinula edodes is the second genera with the highest production in European and Asian markets, and the demand for these mushrooms is constantly rising for their highest chemical composition (Philippoussis et al., 2003; Royse et al., 2017). Over 1,321,000 tons being produced in China, Japan, Taiwan and Korea (Lin et al., 2000; Chang and Miles, 2004). However, the conditions of the cultivation are complicated for some countries because *L. edodes* needs long incubation times and specific substrates to produce fruits body and low temperature (Sánchez-Hernández et al., 2014; Sharma et al., 2015).

Lentinula edodes is a lignocellulolytic fungus, whichs grows on tree trunks, of species such as eucalyptus, oak, mango and avocado (Bach et al., 2018). This specie needs long incubation times, low temperature and specific substrates to produce fruits body such as: sawdust, rice straw, wheat straw, barley straw, vineyard pruning, and

hazelnut husk (Gaitán-Hernández et al., 2006; Valenzuela-Cobos et al., 2017). However, this cultivation system represents a limiting factor and potential danger to the environment due to the slow growth rate and the overuse of the oak, jeopardizing the population of this important forest element (Przybyłowicz and Donoghue, 1990; Valenzuela-Cobos et al., 2019a).

The use of growing substrates and the selection of good strains are of vital importance to ensure a high production of fruit bodies in the shortest possible time. The protein and fat content of the fruit bodies of *L. edodes* depends on the agricultural residues used in the cultivation of the fungus (Harris-Valle et al., 2007; Gaitán-Hernández et al., 2011; Cabrera et al., 2013; Silva et al., 2015; Plotnikov et al., 2016; Valenzuela-Cobos et al., 2017), while the advantages of the development of a hybrid strain are: improving the commercial attributes, decreasing incubation time and using different agricultural wastes. Studies have showed hybrids strains among *Pleurotus djamor* and *L.*

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edodes obtaining fruit bodies with highest productivities and protein contents in relation with the parental strains (Valenzuela-Cobos *et al.*, 2019a). For all these reasons, it is important to study the cultivation of hybrid strains of *L. edodes* on important agricultural residues of Ecuadorian industries such as: peat moss and banana leaves in order to obtain highest productivity parameters and also chemical parameters.

The purpose of this research was to determinate the productivity parameters and the chemical composition of the fruit bodies of parental strains of *L. edodes* and two hybrid strains of *L. edodes* cultivated in two mixtures of agricultural wastes, and also evaluate their antioxidant activities of the ethanolic extracts.

MATERIALS AND METHODS

Biological material

In this experiment was used the following *L. edodes* strains: two native strains (LEN95 and LEN97), two commercial strains (LEC08 and LEC24) and two hybrid strains (LEN95₁XLEC24₂ and LEN95₄XLEC24₁).

The hybrid strains were obtained by pairing compatible neohaplonts of the strains of *Lentinula edodes* LEN95 and LEC24. All the edible fungi strains are maintained on MEA dishes and are deposited at the fungal collection of Research and Development Laboratory of Ecuahidrolizados localized in Guayaquil, Ecuador.

Preparation of malt extract agar (MEA)

The flask with the malt extract agar (MEA) was sterilized at 121 °C for 15 min. After that, 20 mL of MEA were poured into Petri dishes. The dishes with MEA were incubated at 28 °C for 72 h to verify sterility.

Preparation of wheat grain

The wheat grain was hydrated by immersion for 12 hours and sterilized at 121 °C for 60 min. After that, 150 g of wheat grain was placed in bags plastic cylinder (Valenzuela-Cobos *et al.*, 2020).

Substrates preparation

Mushrooms were cultivated in two different mixture of agricultural wastes:

Mixture 1 (M1): mixture of 30% peat moss, 20% banana leaves, 24% oak sawdust, 16% millet seed, 5% cotton seed hull and 5% CaCO₃.

Mixture 2 (M2): mixture of 40% wheat straw, 50% oak sawdust, 5% cotton seed hull and 5% CaCO₃.

The substrates were hydrated, reaching 65% moisture. After that, the substrates were placed (1 kg wet weight) in plastic bags and sterilized for 3.5 h at 121 °C. The ten bags with the mixture were cooled down and the inoculated with 10% (w/w) of wheat grain and incubated in a dark room at 28±2 °C (Valenzuela-Cobos *et al.*, 2019a; Xu *et al.*, 2020).

Induction to form mushrooms

The 10 bags with substrate inoculated with the mycelium were transferred to a dark room for 90 days. After that, the bags were put in the fructification room with the following conditions: relative humidity was maintained between 85 and 90%, temperature of 18±1 °C, air recirculation and period of illumination of 8 h.

Productivity of *Lentinula edodes*

Productivity of the mushroom was evaluated based on the production rate using the parameters of biological efficiency (BE) and total number of production days starting from inoculation (Salmones *et al.*, 1997).

Chemical composition of the fruit bodies

The mushrooms of *L. edodes* obtained in the 3 harvests were dried at 50 °C for 48 h and then milled to perform proximal analysis using official methods. The contents of humidity, ash, fat and protein (N × 4.38) were determined using (AOAC, 2005). The carbohydrates were obtained by using the following equation: Carbohydrates = 100 - (crude protein + fat + ash) (Valenzuela-Cobos *et al.*, 2019b).

Hexanic extraction procedure

The hexanic extract used in the antioxidant activity was taken of the crude fat determination of the fruit bodies of *Lentinula edodes*. Subsequently, the extract was evaporated using a heating plate.

Evaluation of antioxidant activity

The DPPH radical-scavenging activity assay was determined using the methodology of (Mocan *et al.*, 2018).

Statistical analysis

In all analyzes, a completely randomized design and the results were studied using one-way analysis of variance (ANOVA) to determine the significance of individual differences at p<0.05 level, of productivity and chemical composition of the mushrooms of parental and hybrid strains of *L. edodes*, and their antioxidant activities of the hexanic extracts, when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. The analyses were carried out using statistical software (Statgraphic ver. 16).

RESULTS AND DISCUSSION

Productivity parameters

The productivity parameters of the *L. edodes* strains produced using the mixture M1 and the mixture M2 is presented in Table 1.

Hybrid strain of *Lentinula edodes* (LEN95₄XLEC24₁) growth on both mixtures showed the highest productivity parameters (biologic efficiencies between 74.61 and 82.80% and production rates since 1.21 to 1.30%). Parental strains of *Lentinula edodes* cultivated on mixture 1 presented biologic efficiencies ranged from 53.87 to 75.40% and production rates between 0.71 and 1.03%, while parental strains of *Lentinula edodes* cultivated on the mixture 2 showed biologic efficiencies since 50.46 to 64.75% and production rates ranged from 0.77 to 0.91%. For otherwise, the hybrid strain of *Lentinula edodes* (LEN95₁XLEC24₂) cultivated on both mixtures presented biologic efficiencies since 51.65 to 57.86% and production rates ranged from 0.79 to 0.83%.

Valenzuela-Cobos et al., (2019a) reported value of biological efficiency of 79.86% and production rate of 1.31% for one strain of *Lentinula edodes* growth on mixture of oak sawdust, wheat straw, millet seed, cotton seed hull and CaCO₃, while (Gaitán-Hernández et al., 2014) presented values of biological efficiencies ranged from 66.00 to 320.00% and production rates since 0.30 to 3.40% for four strains of *Lentinula edodes* growth in three different mixtures (Control= millet seed *Panicum mileaceum* L.; Formula 1= millet seed, wheat bran, peat moss, CaSO₄; and Formula 2= millet seed, powdered wheat straw, peat moss, CaSO₄). The variability on the productivity parameters observed in this research was significantly influenced by the formulation of the mixture and genetic factors of the different strains.

Chemical composition of the fruiting bodies

Table 2 presents the chemical composition of the fruit bodies of *Lentinula edodes* strains cultivated using the mixture M1 and the mixture M2.

Fruit bodies of the *L. edodes* strain (LEN97) produced on the mixtures M1 and M2 showing the highest moisture contents ranged from 85.10 to 85.90%, whereas the fruiting bodies of the strain LEN95₄XLEC24₁ cultivated on M1 and M2 respectively showing the lowest moisture values since 79.21 to 80.13%. Mushrooms of the strain LEC08 cultivated on the mixtures M1 and M2 presenting the highest fat contents between 1.02 to 1.06%, while the mushrooms of strain LEN95₄XLEC24₁ cultivated on the mixtures M1 and M2 presenting the lowest fat contents since 0.78 to 0.80%

Mushrooms of the hybrid strain LEN95₄XLEC24₁ cultivated on M1 and M2 showing the highest crude protein contents ranged from 16.98 to 17.10%, whereas the fruit bodies of the strain LEN95₁XLEC24₂ produced on both substrates presented the lowest protein values since 13.79 to 14.05%. Otherwise, the mushrooms of strain LEN95 cultivated on the mixtures M1 and M2 presenting the lowest carbohydrate values ranged from 72.51 to 74.44% and also the fruit bodies of the strain LEN97 produced on M2 (74.02%).

Similar results have been reported, (Regula et al., 2007) informed the following chemical composition for one strain of *L. edodes*: protein (17.2%), fat (2.89%), ash (6.73%) and carbohydrates (66%), while (Gaitán-Hernández et al., 2006) presented the following chemical composition for four strains of *L. edodes* cultivated in three different mixtures of agricultural wastes (vineyard pruning, barley straw, and wheat straw): protein contents ranged from 12.37 to 17.19%, ash contents since 3.36 to 6.08%,

Table 1: Productivity parameters of the parental and hybrid strains of *Lentinula edodes* cultivated on different agricultural wastes

Strains	Substrates	Total cultivation time(days)	Biological efficiency(%)	Production rate (%)
LEN95	M1*	67.60±3.14 ^c	66.38±2.03 ^c	0.98±0.09 ^c
	M2	70.40±2.84 ^c	64.27±1.43 ^B	0.91±0.13 ^B
LEN97	M1	73.20±2.07 ^b	75.40±1.29 ^b	1.03±0.15 ^b
	M2	65.80±2.67 ^D	50.46±0.87 ^D	0.77±0.01 ^C
LEC08	M1	80.40±1.86 ^a	60.13±0.97 ^d	0.74±0.04 ^c
	M2	77.90±2.14 ^A	64.75±1.85 ^B	0.83±0.07 ^C
LEC24	M1	75.40±3.12 ^b	53.87±1.27 ^e	0.71±0.10 ^c
	M2	74.30±1.43 ^B	63.74±1.47 ^B	0.86±0.21 ^C
LEN95 ₁ XLEC24 ₂	M1	65.10±0.95 ^c	51.65±0.52 ^e	0.79±0.04 ^c
	M2	69.40±1.18 ^C	57.86±0.93 ^C	0.83±0.12 ^C
LEN95 ₄ XLEC24 ₁	M1	63.50±2.52 ^d	82.80±1.18 ^a	1.30±0.08 ^a
	M2	61.80±1.69 ^E	74.61±0.62 ^A	1.21±0.14 ^A

*M1: 30% peat moss, 20% banana leaves, 24% oak sawdust, 16% millet seed, 5% cotton seed hull and 5% CaCO₃; M2= 40% wheat straw, 50% oak sawdust, 5% cotton seed hull and 5% CaCO₃.

**All values are means ± standard deviation of ten replicates. Lowercase letters indicate difference between productivity of the mushrooms obtained on M1, while uppercase letters indicate difference between productivity of the mushrooms obtained on M2 according to Duncan's test (p < 0.05), n = 10.

Table 2: Chemical composition of the mushrooms of parental and hybrid strains of *Lentinula edodes* cultivated on different agricultural wastes

Strains	Substrates	%Moisture	%Fat	%Crude Protein	%Ash	% Carbohydrate
LEN95	M1*	83.20±1.17 ^b	0.84±0.19 ^d	17.48±0.35 ^a	8.64±0.21 ^a	72.51±2.17 ^c
	M2	82.60±1.35 ^c	0.80±0.04 ^D	15.90±0.42 ^B	8.72±0.04 ^A	74.44±2.37 ^D
LEN97	M1	85.90±0.57 ^a	0.87±0.07 ^c	16.71±0.74 ^a	8.29±0.05 ^a	74.09±2.01 ^b
	M2	85.10±0.09 ^A	0.91±0.05 ^C	17.10±1.15 ^A	7.83±0.12 ^B	74.02±1.74 ^D
LEC08	M1	83.40±0.72 ^b	1.06±0.42 ^a	14.62±0.43 ^c	7.14±0.85 ^b	77.18±3.52 ^a
	M2	84.10±0.74 ^B	1.02±0.15 ^A	13.48±0.71 ^C	6.85±0.42 ^C	78.65±1.04 ^A
LEC24	M1	80.90±1.26 ^c	0.97±0.08 ^b	15.83±0.80 ^b	5.87±0.24 ^c	77.33±2.58 ^a
	M2	81.10±0.12 ^D	0.96±0.24 ^B	15.67±1.18 ^B	6.19±0.36 ^D	77.18±1.47 ^B
LEN95 ₁ XLEC24 ₂	M1	83.76±0.37 ^b	0.83±0.21 ^d	14.05±1.42 ^d	7.54±0.13 ^b	77.58±0.92 ^a
	M2	83.84±0.45 ^B	0.81±0.15 ^D	13.79±0.85 ^D	7.27±0.06 ^B	78.13±1.56 ^A
LEN95 ₄ XLEC24 ₁	M1	79.21±0.07 ^d	0.80±0.04 ^e	17.10±0.12 ^a	7.14±0.38 ^b	74.96±2.01 ^b
	M2	80.13±0.64 ^E	0.78±0.11 ^E	16.98±0.26 ^A	7.08±0.64 ^B	75.16±0.31 ^C

*M1: 30% peat moss, 20% banana leaves, 24% oak sawdust, 16% millet seed, 5% cotton seed hull and 5% CaCO₃; M2: 40% wheat straw, 50% oak sawdust, 5% cotton seed hull and 5% CaCO₃.

**All values are means ± standard deviation of ten replicates. Lowercase letters indicate difference between chemical composition of the mushrooms obtained on M1, while uppercase letters indicate difference between chemical composition of the mushrooms obtained on M2 according to Duncan's test ($p < 0.05$), $n = 10$.

and carbohydrate contents between 75.13 to 82.22%. The use of different substrates offers a nutritious alternative, because mushrooms contain different values of total carbohydrates, proteins and ash (Brisko et al., 2002).

Antioxidant activities of hexanic extracts of *Lentinula edodes*

The antioxidant activities of the hexanic extracts of *L. edodes* mushrooms cultivated on the two mixtures of agricultural wastes were evaluated through radical scavenging activity is presented in the Table 3.

The hexanic extracts of the hybrid strain of *L. edodes* (LEN95₄XLEC24₁) cultivated on both mixtures showed the highest antioxidant effects ($EC_{50} = 8.01$ and 9.84 mg mL^{-1}). Hexanic extracts of mushrooms of parental strains of *L. edodes* cultivated on mixture 1 showed antioxidant activities since 11.25 to 24.13 mg mL^{-1} , while the extracts of mushrooms of parental strains of *Lentinula edodes* cultivated on the mixture 2 presented antioxidant activities ranged from 9.15 to 15.47 mg mL^{-1} . For otherwise, the extracts of the fruit bodies of hybrid strain of *Lentinula edodes* (LEN95₁XLEC24₂) produced on both mixtures presented antioxidant activities between 16.12 and 20.08 mg mL^{-1} .

Diallo et al. (2020) reported values of antioxidant activities using the DPPH since 8.7 to 25.23 mg mL^{-1} for ethanol and aqueous extracts of *Lentinula edodes*, while (Kitzberger et al., 2007) reported value of antioxidant capacity using the DPPH being of 0.25 mg mL^{-1} for ethanolic extract of *Lentinula edodes*. The maintenance of equilibrium between free radical production and antioxidant defenses is an essential condition for normal organism functioning, and an eventual imbalance is reflected through the accumulation

Table 3: Antioxidant activities of the hexanic extracts of the mushrooms of *Lentinula edodes* produced on the two different mixtures

Strains	Substrates	Radical scavenging activity
		DPPH scavenging activity(EC_{50} ; mg mL^{-1})
LEN95	M1*	19.15±1.05 ^b
	M2	14.20±0.12 ^B
LEN97	M1	24.13±0.13 ^a
	M2	15.47±0.85 ^A
LEC08	M1	17.52±0.13 ^c
	M2	9.15±0.27 ^D
LEC24	M1	11.25±0.07 ^d
	M2	10.15±1.02 ^C
LEN95 ₁ XLEC24 ₂	M1	20.08±0.14 ^b
	M2	16.12±0.09 ^A
LEN95 ₄ XLEC24 ₁	M1	9.84±0.12 ^e
	M2	8.01±0.03 ^E

*M1: 30% peat moss, 20% banana leaves, 24% oak sawdust, 16% millet seed, 5% cotton seed hull and 5% CaCO₃; M2: 40% wheat straw, 50% oak sawdust, 5% cotton seed hull and 5% CaCO₃.

**All values are means±standard deviation of three replicates. Lowercase letters indicate difference between the antioxidant activities of the hexanic extracts of the mushrooms of *Lentinula edodes* obtained on M1, while uppercase letters indicate difference between the antioxidant activities of the hexanic extracts of the mushrooms of *Lentinula edodes* obtained on M2 according to Duncan's test ($p < 0.05$), $n=3$.

*The results are presented in EC_{50} values, which means that higher values correspond to lower antioxidant potential. EC_{50} : extract concentration corresponding to 50% of antioxidant activity.

of damaged cell structures (cell membranes, proteins) (Mocan et al., 2018). The use of hybrid strain of *Lentinula edodes* is a positive predictor for the pharmaceutical field.

CONCLUSIONS

The strains of *Lentinula edodes* (LEN97 and LEN95₄XLEC24₁) cultivated on both mixtures produced fruit bodies with highest protein.

The hybrid strain of *Lentinula edodes* (LEN95₄XLEC24₁) cultivated on both mixtures showed the highest biological efficiency and production rate, and also their hexanic extracts presented the highest antioxidant effects.

The use of agricultural wastes such as: peat moss and banana leaves can be used to increase the productivity parameters of *Lentinula edodes*.

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Authors' contributions

Juan Diego Valenzuela-Cobos and Fabricio Guevara-Viejó have developed the experimental plan. Jesennia Cárdenas-Cobo performed the analysis. Rafael Lazo-Sulca and Delia Noriega-Verdugo verified the analytical methods and discussed the results and wrote the final manuscript. María Fernanda Garcés-Moncayo and Ana Grijalva-Endara supervised the findings of this work.

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