Foods 2021

MDPI

Proceedings

Bioconversion of Lemon Basil Straw (*Ocimum Citriodorum* Vis.) Extracts to Mycelium of *Pleurotus Sajor-Caju* (Fr.) Sing. Mushroom in Different Culture Media †

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† Presented at the 2nd International Electronic Conference on Foods, 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Abstract: Lemon Basil Straw (*Ocimum citriodorum* Vis., LBS) is an agricultural waste from production of basil seed. In this work, it was used as a source of bioactive compounds for gray oyster mushrooms (*Pleurotus sajor-caju* (Fr.) Sing.) mycelia growth. The LBS extracts were obtained by submerging the LBS in DI water at atmospheric pressure and various temperatures. Mycelial growth was conducted in solid and liquid media. The experiments were designed within completely randomized design with triplications. This study demonstrated the utilization of LBS extracts to promote the mushroom cultivation.

Keywords: *Pleurotus sajor-caju*; *Ocimum citriodorum*; lemon basil; bioconversion; culture media

Citation: Chanprapai, P.; Sawangkeaw, R. Bioconversion of Lemon Basil Straw (*Ocimum citriodorum* Vis.) Extracts to Mycelium of *Pleurotus sajor-caju* (Fr.) Sing. Mushroom in Different Culture Media. *Proceedings* **2021**, *68*, x.https://doi.org/10.3390/xxxxx

Received: 1 August 2021
Accepted: 22 September 2021
Published: 15 October 2021

1. Introduction

Lemon basil straw (*Ocimum citriodorum* Vis., LBS) is an agricultural waste and it was generally eliminated by burning in the open field that impacts the environment, especially in term of PM2.5. To persuade the farmer from burning the straw, our research group attempted to valorize LBS. For example, the extraction of bioactive compounds by hydrodistillation and supercritical carbon dioxide were successfully demonstrated [1]. The bioconversion is a promising process to utilize the LBS because various bioactive compounds in LBS could promote the desired microbial growth. Besides, some compounds also inhibit the undesired fungi, e.g., *Gliocladium* sp. and *Trichoderma* sp. growth.

Pleurotus sajor-caju (Fr.) Sing. has been extensively cultivated worldwide, including in Thailand. The mushrooms typically perform several secondary metabolites, which have been shown to be excellent antioxidants [2,3]. The alternative substrates contained the bioactive compounds could enhance those secondary metabolites as well. However, it was reported that the excess bioactive compound such as caffeine in the spent coffee grounds might inhibit the mycelial growth [4].

Comparing with common substrate, wood sawdust used in the mushroom cultivation, LBS needs to be milled to the certain particle size before fabricating the cultivation log bag. The reduction of particle size intensively consumes labor, time, and energy. The aim of this study was to utilize the LBS water extracts for the mycelial growth of *P. sajor-*

caju in solid and liquid culture media. To minimize chemical usage, the LBS was submerged in water at difference temperature to extract the bioactive compounds. This method is simple and practical to transfer the knowledge to the lemon basil farmer.

2. Materials and Methods

2.1. Mushroom Collection and Isolation

The fruit body of *P. sajor-caju* was collected from a mushroom farm in The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Thailand. The sporocarps were recorded, identified, and confirmed with morphological characteristics according to the study conducted by Alexopoulos et al. in 1996.

2.2. Plant Extraction

The lemon basil straw (LBS) was collected from Sukhothai Province, Thailand in 2020. The plant was identified by the herbarium of Kasin Suvathabandhu Herbarium. Each 100 g was submerged in 2 L of DI water at 4°C, 25°C, and 121°C for 2 days. All extracts were filtered and kept at 4°C for mycelia growth assay.

2.3. Effects of Mycelial Growth on Solid Media

Four different culture media for mycelial growth were prepared from LBS water extracts and potato broth (1:1, v/v). The composition of testing media is shown Table 1. Mycelial inoculates (5 × 5 mm) were placed on media in triplicates and incubated at room temperature (32 ± 2°C) for 8 days. Linear growth of the mycelium was daily measured by observing mycelial diameter.

Table 1. Composition of testing media.

No.	Treatment	Total (ml)	Ingredient			
			Potato broth (ml)	LBS (ml)	Dextrose (g)	Agar (g)
1	LBS (RT)	100	50	50	2	1.7
2	LBS (121°C)	100	50	50	2	1.7
3	LBS (4°C)	100	50	50	2	1.7
Control	PDA	100	100	0	2	1.7

LBS (RT) is LBS extracted at room temperature and LBS (X°C) is LBS extracted at X°C.

2.4. Effects of Mycelial Production in Liquid Media

Four liquid media including LBS (RT), LBS (121°C), LBS (4°C), and potato broth (PDB) were used in the study. One hundred milliliters of each media were contained in 250-ml in triplicates. The 5×5 mm plug of 6-day mycelial colony was placed on the surface of the liquid medium. The inoculated flasks were then incubated using shaker (115 rpm) in darkness at 30±2°C for 10 days. After incubation, the mycelia were filtered, washed, and dried at 60°C using a hot air oven for determining constant weight of dried mycelia.

2.5. Multiplication of Selected Liquid Media

The optimized liquid media was selected for multiplication or scale-up by shaking flasks (100 ml × 10 flasks per media) comparing with the PDB (control). The testing conditions were temperature of 30 ± 2°C, agitation speed of 115 rpm, and initial pH of 6.5 in darkness for 10 days [5].

2.6. Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) within completely randomized design (CRD) with triplication. Means were also compared by Duncan's multiple range tests (DMRT) and Tukey's tests ($p < 0.05$) running on SPSS program.

3. Results and Discussion

3.1. Isolation of Gray Oyster Mushroom

The isolation of the mushroom was successfully cultured on PDA for 7 days cultivation at room temperature (32 ± 2°C) is revealed in Figure 1. Mushroom mycelium developed from the tissue into the media. The mycelium displayed white in color, high density, and covered on media in 7 days.

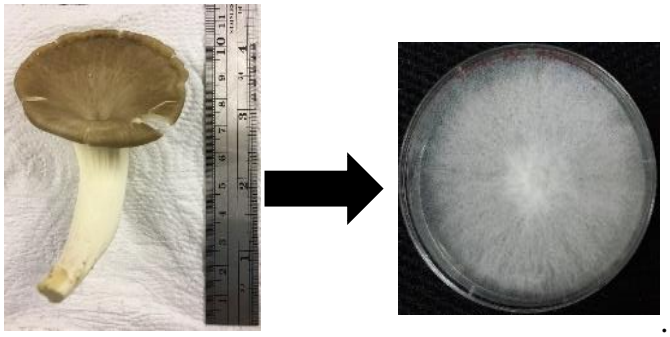

Figure 1. Gray oyster mushroom isolated on PDA media.

3.2. Mycelia Cultivation on Different Solid Media

The mean diameter of mycelium and growth rates are shown in Table 2. The mycelial growth rate was calculated from the linear plot at 8th cultured date. The highest mycelial growth rate of the colony was represented in LBS (RT) media followed by PDA (control), LBS (4 °C) media, and LBS (121 °C) media, respectively. The mycelial growth on LBS (RT) had the fastest growth rate of 12.92 mm^2/day which was 1.5-fold higher than that of the PDA. Thus, it was hypothesized that the bioactive compounds extracted at room temperature (32 ± 2 °C) could promote the growth of mycelium in the solid medium.

Table 2. Mycelial growth of *P. sajor-caju* on different solid media at room temperature (32±2°C).

Treatment	Date and mean diameter of mycelial growth (cm) *							Growth Rate (mm^2/day)	R^2
	2	3	4	5	6	7	8		
LBS (RT)	1.48±.03^a	2.20±.18^a	3.01±.50^a	3.33±.58^a	3.82±.64^a	4.43±.93^a	6.90±.80^a	12.92	0.8945
LBS (121°C)	1.17±.08^b	1.30±.23^c	2.13±.46^b	2.67±.47^b	3.73±.58^a	4.52±.27^a	6.87±.27^a	5.05	0.9157
LBS (4°C)	1.13±.08^b	1.50±.05^{bc}	2.23±.38^b	2.45±.38^b	2.73±.42^b	3.83±.40^a	6.15±.62^a	6.96	0.8495
PDA(Control)	0.97±.15^c	1.67±.15^b	1.92±.08^b	2.10±.10^b	2.50±.10^b	4.17±.29^a	6.37±.15^a	7.85	0.9351
Total							6.57±.57	Sig.=.30	Error=.16
Mean of Square (MS)							0.42	F=1.46	

LBS (RT) is LBS extracted at room temperature and LBS (X°C) is LBS extracted at X°C. * Mean values with different superscript letters in each column are significantly different ($p < 0.05$, DMRT).

3.3. Mycelial Cultivation in Liquid Media

Table 3 depicts the mycelial cultivation of gray oyster mushroom in liquid media (100 ml) using shaking flask (250 ml). PDB medium provided the highest dried biomass of mycelia cultivation. The weights of dried biomass were not difference in LBS (4°C) and LBS (121°C) cultivation, but they were significantly lower than that of the PDB (control). The PDB and LBS (4°C) media were selected for a scale-up. It was found that the dried biomass yields of mycelium growth in PBD and LBS (4°C) media were 3.36 and 2.84 g/l, respectively.

Table 3. Mycelia biomass of *P. sajor-caju* on submerged condition at 30±2°C for 10 days.

Media	Initial pH	Final pH	Fresh biomass (g/100 ml) *	Dried biomass (g/100 ml) *
LBS (RT)	6.50	6.39	3.21±.12^{c}	0.12±.01^{b}
LBS (4°C)	6.50	5.70	8.30±.17^{a}	0.35±.01^{ab}
LBS (121°C)	6.50	6.48	5.10±.17^{b}	0.30±.02^{ab}
PDB (Control)	6.50	5.89	7.98±.22^{a}	0.54±.27^{a}

LBS (RT) is LBS extracted at room temperature and LBS (X°C) is LBS extracted at X°C.* Mean values with different superscript letters in each column are significantly different ($p < 0.05$, DMRT).

4. Conclusions

The mycelial of *P. sajor-caju* mushroom was successfully cultivated on solid and liquid media. The LBS extracted by DI water at room temperature promoted the mycelial growth in solid medium. On the other hand, the LBS extracts from all conditions reduced the weight of dried biomass in the submerged cultivation. This could be the effects of oxygen on the growth of the mycelium with the presence of LBS extracts. Therefore, the water extracted LBS has a high potential to utilize for cultivation of *P. sajor-caju* in the solid medium. Further study on the production of *P. sajor-caju* mushroom spawn on the commercial substrates, e.g., sorghum grain assisting with LBS extract would be interesting to conduct.

Author Contributions: Conceptualization, P.C. and R.S.; methodology, P.C. and R.S.; validation, P.C. and R.S.; formal analysis, P.C.; investigation, P.C.; writing original draft preparation, P.C.; supervision, R.S.; project administration, P.C. and R.S.; funding acquisition, R.S. All authors have read and agreed to the published version of the manuscript.

Funding: The research had been financially supported by the Chulalongkorn University Second Century Fund (C2F) of Postdoctoral Scholarship.

Acknowledgments: The authors would like to thank the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University for working area and equipment.

Conflicts of Interest: The authors declare no conflict of interest.

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