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Research Article

Increasing of Laccase and Manganese Peroxidase Activity by Co-Culture of Immobilized *Pleurotus ostreatus* and *Lentinus tigrinus* Mycelia

Selen Seyide UZAR[1], Ayşe Betül KARADUMAN[2], Mustafa YAMAÇ[3*]

[1] Büyükdere District, Güleryüz Street, No: 21 / 17, Eskisehir, Turkey.
[2] Eskisehir Osmangazi University, Graduate School of Natural and Applied Sciences, Eskisehir, Turkey.
[3] Eskisehir Osmangazi University, Faculty of Science and Arts, Department of Biology, Eskisehir, Turkey.

Abstract:In this study it has firstly reported the using of immobilized fungal mycelia in fungal co-culture studies to enhance activity of lignin-modifying enzymes. For this purpose, the mycelia of *Pleurotus ostreatus* OBCC 6043 and *Lentinus tigrinus* OBCC 3007 were immobilized on nylon scouring pad as carrier material. The immobilized mycelia of the fungi were compared in terms of their laccase and manganese peroxidase activities in mono- and co-culture conditions. The maximum laccase activities of pure *Pleurotus ostreatus* OBCC 6043 and *Lentinus tigrinus* OBCC 3007 cultures were determined as 53.73 and 27.58 U/L, respectively, while the maximum manganese peroxidase activities were 12.54 and 52.02 U/L. In co-culture conditions, distinct enhancement was observed in laccase and manganese peroxidase activities as 319.28 and 554.33 U/L, respectively. In the case of laccase, enzyme activity was 5.94 and 11.58 times higher than that of *Pleurotus ostreatus* OBCC 6043 and *Lentinus tigrinus* OBCC 3007 mono-cultures, respectively. On the other hand, manganese peroxidase activity could be improved distinctly, 44.21 and 10.66 fold higher values than the corresponding ones. The results of the present co-culture study are significantly higher than most of the reported results in the literature, not only for laccase but also for manganese peroxidase activity.

Key words: Immobilization, Laccase, *Lentinus tigrinus*, Manganese peroxidase, *Pleurotus ostreatus*.

Immobilize *Pleurotus ostreatus* ve *Lentinus tigrinus* Misellerinin Birlikte Kültürü ile Lakkaz ve Mangan Peroksidaz Enzim Aktivitelerinin Arttırılması

Öz: Bu çalışmada lignin modifiye edici enzimlerin fazla miktarda üretilmesi için immobilize fungus misellerinin birlikte kültür edilmesi ilk kez olarak rapor edilmiştir. Bu amaçla *Pleurotus ostreatus* OBCC 6043 ve *Lentinus tigrinus* OBCC 3007 miselleri taşıyıcı olarak kullanılan nylon temizlik süngeri üzerine immobilize edilmiştir. Fungusların immobilize miselleri ayrı ayrı ve birlikte kültür edilmeleri durumundaki lakkaz ve mangan peroksidaz aktiviteleri karşılaştırılmıştır.Saf *Pleurotus ostreatus* OBCC 6043 ve *Lentinus tigrinus* OBCC 3007 kültürlerinin maksimum lakkaz aktiviteleri, sırası ile, 53.73 ve 27.58 U/L iken maksimum mangan peroksidaz aktiviteleri 12.54 ve 52.02 U/L olarak belirlenmiştir.

*Corresponding Author: myamac@gmail.com;myamac@ogu.edu.tr

Kokültür koşullarında lakkaz ve mangan peroksidaz aktiviteleri belirgin bir artış ile 319.28 ve 554.33 U/L seviyesine yükselmiştir. *Pleurotus ostreatus* OBCC 6043 ve *Lentinus tigrinus* OBCC 3007 birlikte üretildiklerinde lakkaz aktivitesi ayrı ayrı kültür edilmelerine göre, sırası ile, 5.94 ve 11.58 kat fazla bulunmuştur. Diğer taraftan mangan peroksidaz aktivitesi aynı sıra ile 44.21 ve 10.66 kat gibi belirgin biçimde arttırılabilmiştir. Sunulan birlikte kültür çalışmasının sonuçları, hem lakkaz hem de mangan peroksidaz için literatürde sunulan değerlerin çoğuna göre önemli derecede yüksektir.

Anahtar kelimeler: İmmobilizasyon, Lakkaz, *Lentinus tigrinus*, Mangan peroksidaz, *Pleurotus ostreatus*.

1.Introduction

Saprophytic and/or parasitic fungi especially white rot fungi (WRF) possess some special extracellular enzymes to colonize on dead plant biomass and or alive plant trunk. They are the most efficient colonizer and degrader of the lignocelluloses which is the most abundant biomass form on earth (Dwivedi et al., 2011). Although lignin is a highly recalcitrant polymer, it can be degraded by WRF by their non-specific lignin-modifying enzymes (LMEs) such as lignin peroxidase (LiP, EC 1.11.1.14) manganese peroxidase (MnP, EC 1.11.1.13), laccase (Lac, EC 1.10.3.2) and versatile peroxidase (VP, EC 1.11.1.16). In addition, H_2O_2 generating enzymes (aryl alcohol oxidase, glyoxal oxidase and pyranose-2 oxidase) are considered to be involved in degradation of lignin by WRF (Qi-he et al., 2011).

Xenobiotics refer to compounds that are released into the environment by the action of man and occur in concentrations higher than the natural ones (Ijoma and Tekere, 2017). As a result of their complex structure, most of the xenobiotics are also recalcitrant such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), oil derivatives, pesticides, herbicides, explosives and dyes. Many of xenobiotics have similar structure to lignin. Therefore, WRF are the most efficient degraders of recalcitrant xenobiotics (Kuhar et al., 2015), and can offer an environmental friendly alternative to reduce/solve to the serious environmental pollution. In addition, LMEs from WRF has several industrial applications such as food, brewery and wine processing, wastewater treatment, pulp delignification, biosensor production, the manufacture of anticancer drugs, etc. (Sun and Cheng, 2002; Baldrian, 2006; Rodriguez-Couto and Herrera, 2006; Kunamneni et al., 2008).

Because of their industrial and environmental importance, improving of LMEs production is the focus of many researchers. Optimization of fermentation medium and conditions such as carbon and nitrogen source, pH, oxygen, inducer is the most known and used method to improve LMEs yield (Elisashvili et al., 2008; Flores et al., 2010; Hailei et al., 2013; Jegatheesan et al., 2015; Kuhar et al., 2015). For overproduction of LMEs, several strategies have also been considered including screening of new microorganisms (Okino et al., 2000; Kiiskinen et al., 2004; Machado et al., 2005), using different reactor types (Rivela et al., 2000; Rodriguez-Couto, 2011), oxidative stress by chemicals (Jaszek et al., 2006) or heat-shock (Fink-Boots et al., 1999), and molecular methods such as gene cloning (Kiiskinen and Saloheimo, 2004; Agnieszka et al., 2005; Theerachat et al., 2012; Marková et al., 2016) and UV mutagenesis (D'Souza et al., 2006). Most of these methods are excessively time-, energy- and/or money-consuming. Therefore, more economic, reliable, faster and safer LMEs production method(s) is demanded.

Inoculation or co-culture of another microorganism with a LME producer strain can be a feasible alternative to other methods when their limitations are taken into account.

In the past few decades, some reports have been released regarding co-culture of some LMEs producer fungi such as *Pleurotus* spp. (Vinogradova and Kushnir, 2003; Chi et al., 2007; Dwivedi et al., 2011; Qi-he et al., 2011; Wang et al 2015; Singh et al., 2017), *Trametes* spp. (Hailei et al., 2009; Hiscox et al., 2010; Cupul et al., 2014; Kuhar et al., 2015), *Ganoderma* spp. (Hailei et al., 2013; Kuhar et al., 2015), *Phanerochaete chrysosporium* (Chi et al., 2007; Hu et al., 2011), *Phlebia radiata* (Qi-he et al., 2011; Dong et al., 2012), *Dichomitus squalens* (Qi-he et al., 2011; Dong et al., 2012), *Schizophyllum commune* (Vinogradova and Kushnir, 2003), *Bjerkandera adusta* (Qi-he et al., 2011), *Hypoxylon fragiforme* (Qi-he et al., 2011), *Ceriporiopsis subvermispora* (Dong et al., 2012) and so on. However, up to date, there is not a published study for co-culture of immobilized fungal mycelia to improve LMEs activity. This is the first study that reports the use of immobilized fungal mycelia in co-culture for increasing of laccase and manganese peroxidase activity.

2.Materials and Methods

2.1.Chemicals

Enzyme substrates, DMP (2,6-dimethoxyphenol) and ABTS [2,20-azinobis-(3-ethylbenzthiazoline-6-sulphonate)], and all chemicals used in buffers were obtained from Sigma-Aldrich.

2.2.Fungal Strains

The *Pleurotus ostreatus* strain was kindly donated by Miha Humar (Slovenia) and was coded as OBCC 6043. The strain of *Lentinus tigrinus* OBCC 3007 was obtained from Basidiomycetes Culture Collection in Eskişehir Osmangazi University. It was given a GenBank accession number (MF616404) for the nucleotide sequence of the *Lentinus tigrinus* OBCC 3007. The fungal cultures were stored on Potato Dextrose Agar (PDA) medium and stored 4 ºC. Before the use, the maintained fungi were activated by incubate on fresh PDA medium 7 days at 28 °C.

2.3.Mycelium Immobilization

Five mycelia discs (6 mm diameter) taken from the actively growing margin side of the fungal colony were inoculated to 100 ml Potato Malt Pepton (PMP) medium. After incubation at 28 °C, 100 rpm, 4 days, mycelium was harvested with filtration. Then, total volume was adjusted to 100 ml with sterile distilled water (SDW) and the mycelium was homogenized (Heidolph, Silent Crusher M). Obtained homogenized mycelium suspension was used as inocula 4% for immobilization trials.

Nylon scouring pad (Scotch-Bride) was used as carrier material in immobilization studies (Ibrahim et al. 2014). To prepare it was cut into 1.0 cm^3 cubes, washed twice with methanol, boiled for 10 min, washed twice with SDW, and dried overnight. To perform immobilization, 100 ml fresh PMP medium including appropriate number of scouring pad was inoculated with homogenized mycelium suspension 4%. After incubation of the flasks at 28 °C, 7 days, mycelium immobilized scouring pad discs were collected, washed twice with SDW, and used for inoculation of LMEs production medium for mono- and co-culture studies (Fig 1).

2.4.Culture Conditions

To determine LMEs production capacity of the strains, synthetic fermentation medium (Elisashvili et al., 2008)) was modified in all mono- and co-culture studies with the following composition: Glucose 10 g, wheat bran 1 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, NH_4NO_3 0.5 g, yeast extract 1 g, 1.0% trace element solution included $CaCl_2 . 2H_2O$ 6 g/L, $CuSO_4 . 7H_2O$ 0.5 g/L, $FeSO_4 . 7H_2O$ 0.5 g/L, $MnSO_4 . H_2O$ 0.5 g/L, and $ZnSO_4 . 7H_2O$ 0.1 g/L.

A 500 ml medium was inoculated with 20 mycelium immobilized disc in monoculture studies, while in the case of co-culture 10 mycelium immobilized disc from each species were transferred into 500 ml medium to maintain a total inoculum amount. All of the experimental group flasks were incubated under 100 rpm shaking speed and at 28 °C for a total period of 20 days.

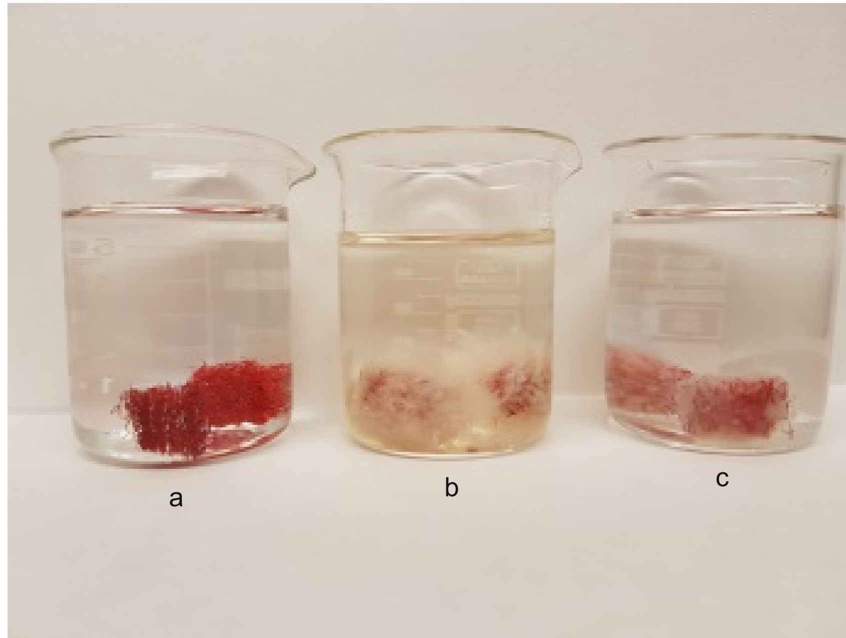

Figure 1. Immobilized fungal mycelia on nylon scouring pad. a. Control, b. *Lentinus tigrinus* OBCC 3007, c. *Pleurotus ostreatus* OBCC 6043

During cultivation, 10 ml samples were taken from the cultures at various times and analysed for their enzymes (laccase and manganese peroxidase) activities, reducing sugar and nitrogen contents of the medium.

2.5. Analytical Methods

Laccase activity was determined as previously reported by Niku-Paavola et al., (1990). Briefly, the increase in A420 due to oxidation of ABTS 0.1 mM (molar extinction coefficient 36000 M^{-1} cm^{-1}) in 100 mM sodium acetate buffer (pH: 4.5) was assayed. MnP activity was also determined in presence of DMP as substrate (molar extinction coefficient 27500 M^{-1} cm^{-1}) and in the condition of A 469, 250 mM sodium tartrate buffer (pH: 4.5) (Ürek and Pazarlıoğlu, 2003). One unit of Lac or MnP activity (U) was defined as the amount of enzyme catalysing oxidation of 1 µmol of the corresponding substrate per minute.

The reducing sugar amount in the sampled culture fluids was assayed with the 3,5-dinitrosalicyclic acid (DNS) method (Miller, 1959). The residual nitrogen amount of the medium was determined with Berthelot method (Searle, 1984)

3. Results and Discussion

It is known that there is no pure culture in any part of natural ecosystems. It means that there is a crucial interaction between not only fungal species but also all different microorganism groups, which are living in same habitats.

Bioconversion of lignocellulosic biomass into its components has a major importance for recycling of the carbon fixed by photosynthesis. Most of the natural degradation processes occur by the combination of enzyme mixtures and metabolic pathways of several microorganisms (Hu et al., 2011; Dong et al., 2012). Therefore, co-culture of different microorganism culture may result in production of the preferred enzymes more efficiently than their monocultures (Hu et al., 2011). By far, several reports have been released to fungal co-culturing to increase production of the enzymes involving in lignocellulose degradation.

Although there are several reports for cellulase (Vinogradova and Kushnir, 2003; Hu et al., 2011; Singh et al.; 2017), xylanase (Dwivedi et al., 2011; Hu et al., 2011; Singh et al.; 2017) and LiP (Qi-he et al., 2011; Dong et al., 2012), majority of the attempts to increase laccase (Hailei et al., 2009, 2013; Flores et al., 2010; Hiscox et al., 2010; Hu et al., 2011; Qi-he et al., 2011; Dwivedi et al., 2011; Dong et al., 2012; Cupul et al., 2014; Kuhar et al., 2015; Wang et al., 2015; Singh et al.; 2017). On the other hand, Cupul et al., (2014) reported that induction of MnP production in co-culture conditions has been paid relatively little attention.

In the presented study, immobilized *P. ostreatus* and *L. tigrinus* mono- and co-cultures were compared for their time dependent LMEs activities. . In the pure cultures of *P. ostreatus*, the maximum laccase and MnP activities were determined as 53.73 and 12.54 U/L on the days 20 and 17 of incubation, respectively (Fig 2). On the other hand the activities of the same enzymes by *L. tigrinus* were presented as 27.58 and 52.02 U/L on the 9th day (Fig 3). As it can be seen from figures 2 and 3, the time for maximal Lac and MnP activity by *L. tigrinus* was at least 8 days shorter and MnP activity was 4.15 times higher than *P. ostreatus*. But the Lac activity of the *P. ostreatus* was 1.95 fold that of *L. tigrinus* activity.

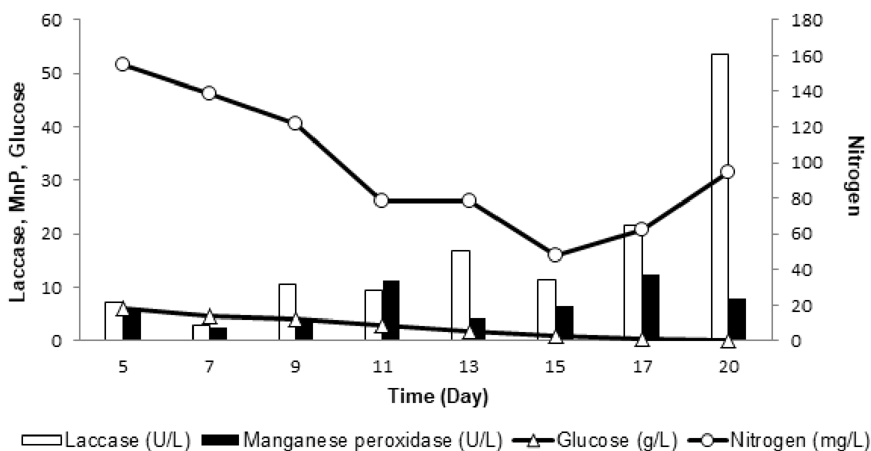

Figure 2. Time course lignin-modifying enzyme activity of *Pleurotus ostreatus* mono-culture

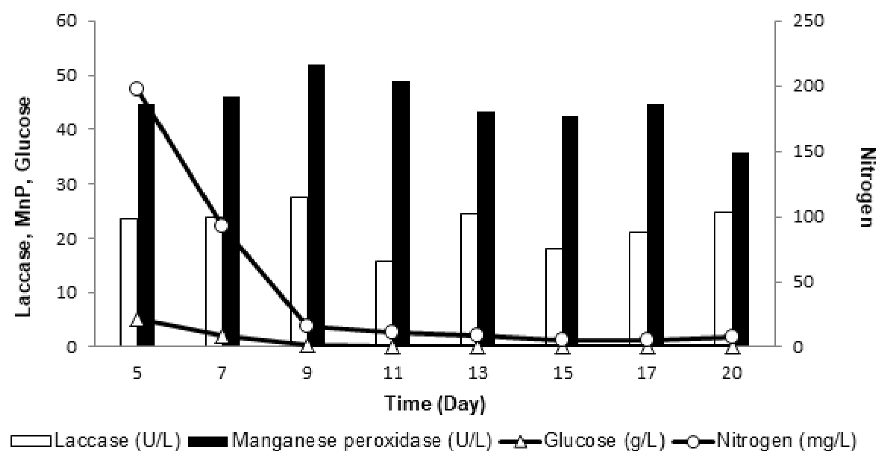

Figure 3. Time course lignin-modifying enzyme activity of *Lentinus tigrinus* mono-culture

Overall, it is clearly seen that the use of co-cultures appears to be more successful for enzyme activity values (Fig. 4). Distinct enhancement was observed in the level of both enzymes under co-cultivation condition. The lac activity of co-culture (319.28 U/L) was 5.94 and 11.58 times higher than that of *P. ostreatus* and *L. tigrinus*, respectively. In the case of MnP, the mixed culture (554.33 U/L) was presented 44.21 and 10.66 times higher values than the corresponding ones.

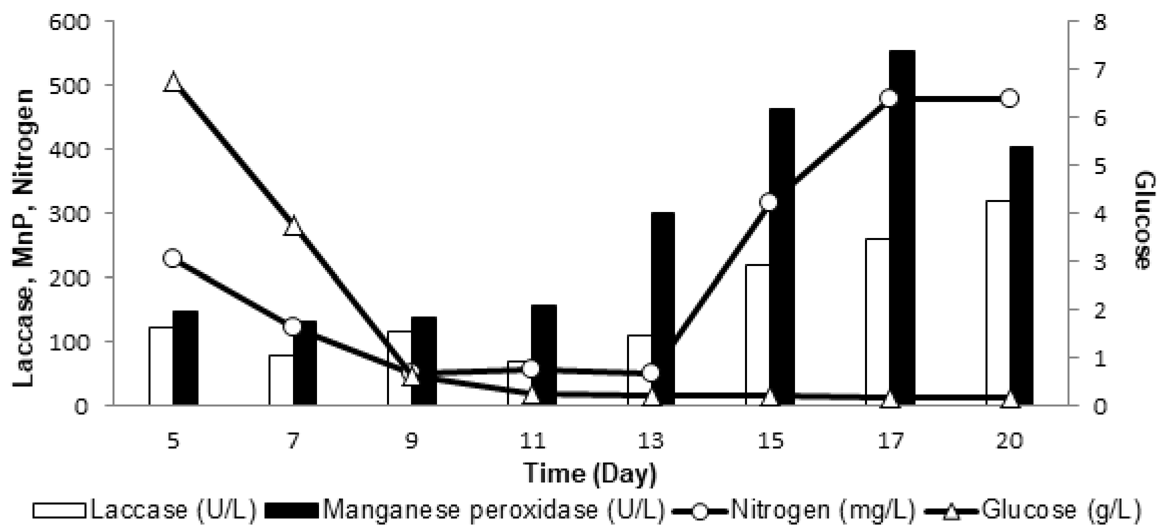

Figure 4. Time course lignin-modifying enzyme activity of *Pleurotus ostreatus* and *Lentinus tigrinus* co-culture

It is important to note that Lac and MnP activities of the co-culture were 3.93 and 8.59 fold higher than total Lac and MnP activities of both of the fungi. A possible explanation for these impressive enzyme activities in co-culture conditions is synergistic interaction for the studied fungi.

Dwivedi et al. (2011) informed that the interactions between fungi might be antagonistic, parasitic or synergistic during co-culture of the fungi. Antagonistic and synergistic interactions could have beneficial effets on the LMEs production as it triggers a switch to secondary metabolism (Baldrian, 2004; Chi et al., 2007; Hiscox et al., 2010). But this effect is species specific (Ijoma and Tekere, 2017) and strain compatibility has been reported as the most determining factor for successful co-culturing (Singh et al., 2017). In view of these reports, it can be argued that the used *P. ostreatus* and *L. tigrinus* strains were found to be compatible for their co-culture for LMEs activity. Singh et al., (2017) reported that if the studied strains are compatible, there is a probability for synergy between metabolic pathway of involved strain. Although some reports has highlighted to the failed stimulation for LMEs activity (Koroleva et al., 2002; Elisashvili and Kachlishvili, 2009), LMEs activity could be enhanced by most of the fungal co-culture studies (Table 1).

As it can be seen from Table 1, the results of the present co-culture study are distinctly higher than most of the reported ones for not only laccase but also MnP activity. It is worth mentioning that increasing ratio of MnP is uniqe according to level of the *P. ostreatus*.

Table 1. Co-culture studies for *lignin modifying enzyme production*

Organisms	*Enzyme*	*Enzyme activity**			*Culture* **	*Reference*
		Mono-culture	*Co-culture*	Fold		
Aspergillus niger - Phanerochaete chrysosporium	Laccase	1.30 - 0.20 [b]	3.10 [b]	2.38 - 15.5	SmF	Hu et al., 2011
Aspergillus oryzae - Phanerochaete chrysosporium		1.80 - 0.20 [b]	3.60 [b]	2.00 - 18.00	SmF	
Ceriporiopsis subvermispora - Pleurotus ostreatus		0.70 - 1.50 [b] ***	2.20 [b] ***	3.14 - 1.47	SmF	Chi et al., 2007
Phlebia radiata, - Ceriporiopsis subvermispora		20.00 - Ø [b] ***	95.00 [b] ***	4.75 - Ø	SmF	Dong et al., 2012
Phlebia radiata - Dichomitus squalens		80.00 - 30.00 [c] ***	120.00 [c]	1.50 - 4.00	SmF	Qi-he et al., 2011
Pleurotus ferulae - Rhodotorula mucilaginosa		3.74 - Ø [b]	10.58 [b]	2.83 - Ø	SmF	Wang et al., 2015
Pleurotus ostreatus - Lentinus tigrinus		53.73 - 27.58 [d]	319.28 [d]	5.94 – 11.58	SmF	This study
Pleurotus ostreatus - Penicillium oxalicum		13.02 - Ø [b]	20.83 [b]	1.60 - Ø	SmF	Dwivedi et al., 2011
		13.02 - Ø [b]	15.00 [b]	1.15 - Ø	SF	
		13.02 - Ø [b]	43.70 [b]	3.36 - Ø	SSF	
Pleurotus ostreatus - Trichoderma viridae		20.80 - Ø [d]	55.00 [d]	2.64 - Ø	SmF	Flores et al., 2010
		2.00 - Ø [a]	10.00 [a]	5.00 - Ø	SSF	Singh et al., 2017
Trametes maxima - Paecilomyces carneus		4881.07 - Ø [c]	12382.50 [c]	2.54 - Ø	SmF	Cupul et al., 2014
Trametes versicolor - Ganoderma lucidum		0.86 - 2.24 [a]	7.93 [a]	9.22 - 3.54	SSF	Kuhar et al., 2015
Trametes versicolor - Bjerkandera adusta		216.00 - Ø [f]	871.6 [f]	4.04 - Ø	Agar	Hiscox et al., 2010
Trametes versicolor - Daldinia concentrica		216.00 - Ø [f]	877.80 [f]	4.06 - Ø	Agar	Hiscox et al., 2010
Trametes versicolor - Fomes fomentarius		216.00 - Ø [f]	141.80 [f]	- 1.52	Agar	Hiscox et al., 2010
Trametes versicolor - Hypholoma fasciculare		216.00 - 31.4 [f]	417.10 [f]	1.93 - 13.28	Agar	Hiscox et al., 2010
Trametes versicolor - Stereum gausapatum		216.00 - 27.30 [f]	3218.90 [f]	14.90 - 117.90	Agar	Hiscox et al., 2010
Trametes versicolor - a yeast strain		0.89 - Ø [b]	10.50 [b]	11.80 - Ø	SmF	Hailei et al., 2009

* a: U/g, b: U/ mL, c: u/mg protein, d: U/L, e: nkat/L, f: mU / g wet weight

** SSF: Solid state fermentation, SmF: Submerged fermentation

*** These data were extracted from the graphics in the references.

Table 1. Continued

Organisms	*Enzyme*	*Enzyme activity**			*Culture***	*Reference*
		Mono-culture	*Co-culture*	Fold		
Ceriporiopsis subvermispora - Pleurotus ostreatus	MnP	100.00 - 20.00 [e] ***	140.00 [e] ***	1.40 - 7.00	SmF	Chi et al., 2007
Physisporinus rivulosus - Pleurotus ostreatus		35.00 - 20.00 [e] ***	250.00 [e] ***	7.14 - 12.50	SmF	Chi et al., 2007
Phlebia radiata - Dichomitus squalens		25.00 - 18.00 [b] ***	55.00 [b] ***	2.20 - 3.06	SmF	Dong et al., 2012
Pleurotus ostreatus - Lentinus tigrinus		12.54 - 52.02 [d]	554.33 [d]	44.21 - 10.66	SmF	This study
Pleurotus ostreatus - Phlebia radiata		350.00 - 100.00 [e] ***	800.00 [e] ***	2.28 - 8.00	SmF	Qi-he et al., 2011
Trametes maxima - Paecilomyces carneus		291.80 - Ø [c]	564.12 [c]	1.93 - Ø	SmF	Cupul et al., 2014
Trametes versicolor - Stereum gausapatum		Ø - 2.70 [f]	33.60 [f]	Ø - 12.44	Agar Medium	Hiscox et al., 2010
Trametes versicolor - Daldinia concentrica		Ø - Ø [f]	10.40 [f]	10.40	Agar Medium	Hiscox et al., 2010
Trametes versicolor - Bjerkandera adusta		Ø - 3.90 [f]	11.00 [f]	Ø - 2.82	Agar Medium	Hiscox et al., 2010
Trametes versicolor - Fomes fomentarius		Ø - Ø [f]	5.90 [f]	5.90	Agar Medium	Hiscox et al., 2010
Trametes versicolor - Hypholoma fasciculare		Ø - 8.90 [f]	8.70 [f]	- 1.02	Agar Medium	Hiscox et al., 2010
Pleurotus ostreatus - Phlebia radiata	LiP	160.00 - Ø [e] ***	60.00 [e] ***	- 2.66	SmF	Qi-he et al., 2011
Aspergillus flavus - Trichoderma viridae	Cellulase	8.20 - 6.70 [a]	11.00 [a]	1.34 - 1.64	SSF	Singh et al., 2017
	Xylanase	72.00 - 80.00 [a]	180.00 [a]	2.50 - 2.25	SSF	Singh et al., 2017
Pleurotus ostreatus - Penicillium oxalicum		16.00 - 714.13 [b]	1201.84 [b]	75.12 - 1.68	SmF	Dwivedi et al., 2011
		16.00 - 714.13 [b]	976.55 [b]	61.03 - 1.37	SF	Dwivedi et al., 2011
		16.00 - 714.13 [b]	1301.51 [b]	81.34 - 1.82	SSF	Dwivedi et al., 2011

Possible reasons of the increase in of the fungal enzyme activities in co-culture conditions were reported as competition for space and nutrients (Asiegbu et al., 1996; Qi-he et al., 2011), synergistic interactions (Bader et al., 2010; Dong et al., 2012), glucose starvation (Hailei et al., 2009), oxidative stress (Jaszek et al., 2006; Chi et al., 2007) and supplementation of other carbon source and inducers (Hailei et al., 2009; Li et al., 2011). Depending of the results of this study, we have suggested a synergistic interaction between the studied *L. tigrinus* and *P. ostreatus* strains. The laccase and MnP enzyme production level of the studied mushroom species are promising for not only enzyme but also basidiomata production of these edible mushroom species.

Immobilization of the fungal mycelium appears to be an attractive approach to protect the mycelium from the environmental stress factors during fermentation. Besides it is a non-toxic, economic, easy, fast and reusable alternative to obtain the best performance of the fungus. In this study, nylon scouring pad was preferred as carrier since this carrier provides novel living space for the fungal mycelium to grow and produce enzyme (Ibrahim et al. 2014).

As a consequence, it could be concluded that co-culturing of these two compatible mushroom species may have potential for cost effective enzyme production, co-production of their basidiomata production and/or basidiomata production of one of these species on spent compost of the other one. Our further investigations will be focused on the scaling-up of the enzyme production process and determination of the co-culture conditions for basidiomata production of *L. tigrinus* and *P. ostreatus* strains.

References

Agnieszka K., Christian G., Marcel A., André F., Marius R., Thierry T,. *LAC3, a new low redox potential laccase from Trametes sp. strain C30 obtained as a recombinant protein in yeast*, Enzyme Microb. Technol., 36, 34-41, (2005).

Asiegbu F.O., Paterson A., Smith, J.E., *The effects of co-fungal cultures and supplementation with carbohydrate adjuncts on lignin biodegradation and substrate digestibility*, World J. Microbiol. Biotechnol., 12, 273–279, (1996).

Bader J., Mast-Gerlach E., Popović M.K., Bajpai R., Stahl U., *Relevance of microbial coculture fermentations in biotechnology*, J. Appl. Microbiol., 109(2), 371-387, (2010).

Baldrian P., Increase of laccase activity during interspecific interactions of white-rot fungi, FEMS Microbiol. Ecol., 50, 245-253, (2004).

Baldrian, P., *Fungal laccases – occurrence and properties*, FEMS Microbiol. Rev., 30, 215–242, (2006).

Chi Y., Hatakka A., Maijala P., *Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes?* Int. Biodeter. Biodeg., 59, 32–39, (2007).

Cupul W.C., Abarca G.H., Carrera D.M., Vázquez R.R., *Enhancement of ligninolytic enzyme activities in a Trametes maxima–Paecilomyces carneus co-culture: Key factors revealed after screening using a Plackett–Burman experimental design*, Electr. J. Biotechnol., 17, 114–121, (2014).

Dong Y.C., Wang W., Hu Z.C., Fu M.L., Chen Q.H., *The synergistic effect on production of lignin-modifying enzymes through submerged co-cultivation of Phlebia radiata, Dichomitus squalens and Ceriporiopsis subvermispora using agricultural residues*, Bioprocess Biosyst. Eng., 35, 751–760, (2012).

D'Souza D.T., Tiwari R., Sah A.K., Raghukumar C., *Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthesis dyes*, Enzyme Microb. Technol., 38, 504–511, (2006).

Dwivedi P., Vivekanand V., Pareek N., Sharma A., Singh R.P., *Co-cultivation of mutant Penicillium oxalicum SAUE-3.510 and Pleurotus ostreatus for simultaneous biosynthesis of xylanase and laccase under solid-state fermentation*, New Biotechnol., 28 (6), 616-626, (2011).

Elisashvili V., Kachlishvili E., *Physiological regulation of laccase and manganese peroxidase production by white-rot Basidiomycetes*, J. Biotechnol., 144, 37-42, (2009).

Elisashvili V., Kachlishvili E., Penninckx M., *Effect of growth substrate, method of fermentation, and nitrogen source on lignocellulose-degrading enzymes production by white-rot basidiomycetes*, J. Ind. Microbiol. Biotechnol., 35, 1531–1538, (2008).

Fink-Boots M., Malarczyk E., Leonowicz A., *Increased enzymatic activities and levels of superoxide anion and phenolic compounds in cultures of basidiomycetes after temperature stress*, Acta Biotechnol., 19, 319–330, (1999).

Flores C., Casasanero R., Trejo-Hernandez M.R., Galindo E, Serrano-Carreo L., *Production of laccases by Pleurotus ostreatus in submerged fermentation in co-culture with Trichoderma viride*, J. Appl. Microbiol., 108, 810–817, (2010).

Hailei W., Guangli Y., Ping L., Yanchang G., Jun L., Guosheng L., Jianming Y., *Overproduction of Trametes versicolor laccase by making glucose starvation using yeast*, Enzyme Microbial Technol., 45, 146–149, (2009).

Hailei W., Chaozhi T., Guangli Y., Ping L., *A novel membrane-surface liquid co-culture to improve the production of laccase from Ganoderma lucidum*, Biochem. Eng. J., 80, 27– 36, (2013).

Hiscox J., Baldrian P., Rogers H.J., Boddy L., *Changes in oxidative enzyme activity during interspecific mycelial interactions involving the white-rot fungus Trametes versicolor*, Fungal Gen. Biol., 47, 562–571, (2010).

Hu H.L., van den Brink J., Gruben B.S., Wösten H.A.B., Gu J.D., de Vries R.P*., Improved enzyme production by co-cultivation of Aspergillus niger and Aspergillus oryzae and with other fungi*, Int. Biodeter. Biodeg., 65, 248-252, (2011).

Ibrahim D., , Weloosamy H., Sheh-Hong L., Potential use of nylon scouring pad cubes attachment method for pectinase production by *Aspergillus niger* HFD5A-1, Process Biochemistry, 49, 660-667, (2014),

Ijoma G.N., Tekere M., *Potential microbial applications of co-cultures involving ligninolytic fungi in the bioremediation of recalcitrant xenobiotic compounds*, Int. J. Environ. Sci. Technol., 14(8), 1787–1806, (2017).

Jaszek M., Grzywnowicz K., Malarczyk E., Leonowicz A., *Enhanced extracellular laccase activity as a part of the response system of white rot fungi Trametes versicolor and Abortiporus biennis to paraquat-caused oxidative stress conditions*, Pestic. Biochem. Physiol. 85, 147–154, (2006).

Jegatheesan M., Eyini M., *Response Surface Methodology Mediated Modulation of Laccase Production by Polyporus arcularius*, Arab. J. Sci. Eng., 40, 1809–1818, (2015).

Kiiskinen L.L., Saloheimo M., *Molecular cloning and expression in Saccharomyces cerevisiae of a laccase gene from the ascomycete Melanocarpus albomyces*, Appl. Environ. Microbiol., 70, 137–144, (2004).

Kiiskinen L.L., Rättö M., Kruus K., *Screening for novel laccase-producing microbes*, J. Appl. Microbiol., 97, 640–646, (2004).

Koroleva O.V., Stepanova E.V., Gavrilova V.P., Yakovleva N.S., Landesman E.O., Yavmetdinov I.S., Yaropolov A.I., *Laccase and Mn-peroxidase production by Coriolus hirsutus strain 075 in a jar fermentor*, J. Biosci. Bioeng., 93, 449-455, (2002).

Kuhar F., Castiglia V., Levin L., *Enhancement of laccase production and malachite green decolorization by co-culturing Ganoderma lucidum and Trametes versicolor in solid-state fermentation*, Int. Biodeter. Biodeg., 104, 238-243, (2015).

Kunamneni A., Plou F.J., Ballesteros A., Alcalde M., *Laccases and their applications: a patent review*, Recent Pat. Biotechnol., 2, 10–24, (2008).

Li P., Wang H.L., Liu G.S., Li X., Yao J.M., *The effect of carbon source succession on laccase activity in the co-culture process of Ganoderma lucidum and a yeast*, Enzyme Microb. Technol., 48, 1–6, (2011).

Machado K.M.G., Matheus D.R., Bononi V.L.R., *Ligninolytic enzymes production and Remazol Brilliant Blue R decolorization by tropical brazilian basidiomycetes fungi*, Brazilian J. Microbiol., 36, 246-252, (2005).

Marková E., Kotik M., Křenková A., Man P., Haudecoeur R., Boumendjel A., Hardré R., Mekmouche Y., Courvoisier-Dezord E., Réglier M., Martínková L., *Recombinant tyrosinase from Polyporus arcularius: Overproduction in Escherichia coli, Characterization, and Use in a Study of Aurones as Tyrosinase Effectors,* J. Agric. Food Chem.., 64(14), 2925-2931, (2016).

Miller G.L., *Use of dinitrosalicylic acid reagent for determination of reducing sugar*, Anal. Chem., 31, 426-428, (1959).

Niku-Paavola M., Raaska L., Itävaara M., *Detection of white-rot fungi by a non-toxic stain*, Mycol. Res., 94, 27-31, (1990).

Qi-he C., Krügener S., Hirth T., Rupp S., Zibek S., *Co-cultured Production of Lignin-Modifying Enzymes with White-Rot Fungi*, Appl. Biochem. Biotechnol., 165,700–718, (2011).

Okino L.K., Machado K.M.G., Fabris C., Bononi V.L.R., *Ligninolytic activity of tropical rainforest basidiomycetes*, World J. Microbiol. Biotechnol., 16, 889-893, (2000).

Rivela I., Rodriguez-Couto S., Sanroman A., *Extracellular ligninolytic enzyme production by Phanerochaete chrysosporium in a new solid-state bioreactor*, Biotechnol. Lett., 22, 1443-1447, (2000).

Rodriguez-Couto S., *Production of laccase and decolouration of the textile dye Remazol Brilliant Blue R in temporary immersion bioreactors*, J. Hazard. Mat., 194, 297–302, (2011).

Rodriguez-Couto S., Herrera, J.L.T., *Industrial and biotechnological applications of laccases: a review*, Biotechnol. Adv., 24, 500–513, (2006)

Searle P., *The berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen*. A review, Analyst., 109, 549–568, (1984).

Singh N., Devi A., Jaryal R., Rani K., *An Ecofriendly and Efficient Strategy for Cost Effective Production of Lignocellulotic Enzymes*, Waste Biomass Valor., DOI 10.1007/s12649-017-9861-9, (2017).

Sun Y., Cheng J., *Hydrolysis of lignocellulosic material from ethanol production: a review*, Bioresour. Technol., 83, 1–11, (2002).

Theerachat M., Emond S., Cambon E., Bordes F., Marty A., Nicaud J.M., Chulalaksananukul W., Guieysse D., Remaud-Siméon M., More S., *Engineering andproduction of laccase from Trametes versicolor in the yeast Yarrowia lipolytica*, Bioresour. Technol., 125, 267–274, (2012).

Ürek R.Ö., Kasikara Pazarlıoğlu N., *Purification and partial characterization of manganase peroxidase from immobilized Phanarochaete chrysosporium*, Process Biochem., 39, 2061-2068, (2003)

Vinogradova S.P., Kushnir S.N., *Biosynthesis of Hydrolytic Enzymes during Cocultivation of Macro- and Micromycetes*, Appl. Biochem. Microbiol., 39(6), 573–575, (2003).

Wang H., Peng L., Ding Z. , Wu J., Shi G., *Stimulated laccase production of Pleurotus ferulae JM301 fungus by Rhodotorula mucilaginosa yeast in co-culture*, Process Biochem., 50, 901–905, (2015).