https://bjm.ui.ac.ir/?lang=en

Journal of Microbial Biology
E-ISSN: 2322-5173
12rd Year, Vol. 12, No. 48, Winter 2023 pp. 17-26
Received: 19-04-2023 Accepted: 17-07-2023

**(Research Paper)**

# Improvement of Laccase Production by the Co-culture *Pleurotus florida* and *Rhodotorula mucilaginosa* in Submerged Fermentation Culture

**Seyed Javad Sanei**
Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, sa_nei@yahoo.com

**Seyed Esmaeil Razavi***
Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, razavi@gau.ac.ir

**Abdolhossein Taheri**
Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, a.taheri@yahoo.com

## Abstract

**Introduction:** Extracellular laccases are constitutively formed in fungi, particularly from basidiomycetes, during secondary metabolism. However, the enzymes are produced in relatively small amounts. Due to their wide application, the productivity improvement process of laccase is important for potential industrial applications. Interspecific interaction of lignolytic fungi with other fungi or bacteria is a technique to improve the production of laccase in a liquid state.

**Materials and Methods:** In this research, the interspecific interaction of a yeast, *Rhodotorula mucilaginosa*, with white-rot fungi, *Pleurotus florida*, was evaluated in submerged fermentation using potato dextrose broth. The yeast cells at $10^3$, $10^5$, and $10^7$ CFU $mL^{-1}$ concentrations were added into 1-, 3-, 5- and 8-day cultures of *P. florida*. To investigate the effect of temperature on *R. mucilaginosa* cells or its metabolites for laccase enhancement, yeast cells were exposed to different temperatures (1 h), including room temperature (control), 70 ˚C, and 121 ˚C (autoclaved). Then, 3% of the suspension (v/v) was added to the *P. florida* culture. The laccase activity was assessed by the colorimetric method at 436 nm.

*Corresponding Author



Sanei S. J., Razavi S. E. and Taheri A. Improvement of Laccase Production by the Co-culture Pleurotus florida and Rhodotorula mucilaginosa in Submerged Fermentation Culture. *Journal of Microbial Biology* 2023; 12 (48): 17-26.
http://dx.doi.org/10.22108/bjm.2023.137389.1535

**Results:** The results showed that, in comparison to control, the laccase activity was enhanced 4.5 times during *P. florida* yeast interactions in potato dextrose broth medium. Production of the enzyme was significantly affected by the yeast cell concentration and the inoculation time of *R. mucilaginosa* in the co-culture of *P. florida*. Maximum enzyme production was achieved when the 5-day *P. florida* culture inoculated with $10^5$ CFU $mL^{-1}$ of *R. mucilaginosa*. The addition of autoclaved (121 ˚C) yeast cells to *P. florida* culture did not significantly increase laccase production as compared to control (monocultures of *P. florida*), although the lowest sterilization temperature (70 ˚C) had a stimulatory effect on laccase production.

**Discussion and Conclusion:** The results of the study showed the capability of yeast to increase the laccase production by *P. florida* in dual cultures. The responses of the laccase production could be affected by the inoculation time (after *P. florida* cultivation) and *R. mucilaginosa* cell concentration. The interactions needed the live stimulator cells and the stimulatory compounds were temperature-sensitive.

**Key words:** Co-cultivation, Culture Extract, White-rot Mushroom, Laccase, Yeast

## Introduction

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10. 3.2) is a phenol-oxidizing enzyme that oxidizes a large variety of organic substrates including aromatic and phenolic compounds with the reduction of oxygen (1). Due to its multiple physiological functions such as detoxification, lignin degradation, plant pathogenesis, and stress defense, laccase is broadly used in various biotechnological processes, including textile bleaching (2), pulps delignification (3), effluent detoxification (4), and in various food industry processes (5).

Laccases are commonly distributed in bacteria, higher plants, and insects; however, fungi, particularly basidiomycetes, are a more important source. Under natural conditions, low constitutive extracellular laccases from white-rot fungi cannot relieve the practical demands of industrial biotechnology. Therefore, it is crucial to improve the productivity process for potential industrial and biotechnological applications (6).

Enhancing fungal laccase production has been obtained by optimizing different nutritional (mainly carbon and nitrogen sources) and physicochemical (temperature, pH, yeast cell concentration, and agitation rates) conditions (7). Much consideration has also been performed for laccase enhancement by different inducers such as phenolic compounds (8), copper (9), and ethanol (10). Oxidative stress is also proposed as a mechanism for laccase overproduction by an inducer (11).

The cultivation of white-rot fungi with different microorganisms (mixed fermentation) is of interest for their ability to induce enzyme activity (12). Enhanced laccase production was effectively reported by white-rot fungi during interaction with *Trichoderma* spp. or its culture metabolite (13, 14) and yeasts (15). Co-culture with yeasts led to glucose deprivation, which caused the overproduction of *Ganoderma lucidum* (12), *Pleurotus eryngii* var. *ferulae* (16), and *T. versicolor* (11) laccase. The purpose of this study was to increase laccase production by *Pleurotus florida*, a commercial edible oyster mushroom by *R. mucilaginosa*, as a biotechnologically important yeast. In addition, we identified the effect of yeast cell concentration and time of *R. mucilaginosa* inoculation in the co-cultured media.

http://dx.doi.org/10.22108/bjm.2023.137389.1535

## Materials and Methods

**Chemicals and Media:** ABTS (>98.0%), potato dextrose agar (PDA), and potato dextrose broth (PDB) media were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Organisms:** *P. florida* strain 285 (gau.1999.511) and *R. mucilaginosa* (gau.1400.222) were kindly provided by the culture collection of the Department of Plant Protection, Gorgan University of Agricultural Sciences and Natural Resources. The fungal strains were maintained on PDA slants at 5-7 °C in the dark.

**Culture Conditions:** The submerged fermentation (SmF) was used for the determination of maximum laccase production at 0-20 day intervals (17). The cultures were performed in 250 mL flasks with 50 mL of PDB medium. The SmF cultures were inoculated with four fungal agar discs (5 mm, diameter) of the 8-day culture and incubated at 25±2 °C, in the dark for 14 days.

**Co-culture of *P. florida* with yeast and extraction of yeast cells:** The 48-h-old culture of *R. mucilaginosa* in PDB was used for inoculation of *P. florida* cultures. The yeast cell concentrations at $10^3$, $10^5$, and $10^7$ CFU $mL^{-1}$ were added into 1-, 3-, 5- and 8-day cultures of *P. florida* to estimate the effect of inoculation size and time on the improvement of laccase production. Yeast cells were collected by centrifugation and their number was determined using a hemocytometer. To investigate the effect of temperature on *R. mucilaginosa* cells or its metabolites for laccase enhancement, yeast cells were exposed to different temperatures for 1 h, including 121 ◦C (autoclaved) and 70 ◦C, and 3% of the suspension (v/v) was added into the *P. florida* culture (18).

**Biochemical Analyses:** The laccase activity was assessed by the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonnic acid) (ABTS) as the substrate. A reaction mixture containing 0.5 mM ABTS, 2.8 mL sodium acetate buffer 0.1 M (pH 4.5), and 100 µL of culture supernatant was incubated for 5 min. The absorbance was determined at 436 nm and one unit (U) of enzyme activity was defined as one µmol of substrate that is oxidized per min (19).

**Statistical Analysis:** All the experimental data were analyzed as factorial using a randomized complete design with four replications. A two-way ANOVA *was* used to analyze the data, and differences between means were compared using Duncan's multiple range tests at $P \leq 0.05$. Regression models were also used to predict the influence of the independent variable, namely temperatures on laccase production in *P. florida* cultures. All statistical analyses were performed using R 4.2.1 statistical software.

## Results

Analysis of variance revealed significant differences in the laccase production by *P. florida* on different days ($P \leq 0.05$, Table 1). The laccase production by *P. florida* dramatically increased at various time intervals (Fig. 1). The highest laccase activity in the PDB medium (2.88 U $mL^{-1}$) was obtained on the 14th day of cultivation and became partially constant thereafter. The relationship between time (x) and maximum laccase activity (y) was described by $y=-0.0101x^2 + 0.3881x - 0.7937$ model (adjusted R-squared = 0.9738 and $p<0.001$). Considering the results, the effects of *R. mucilaginosa* on laccase activity were screened on the 14th day. Laccase activity was not detected in *R. mucilaginosa* cultures.

http://dx.doi.org/10.22108/bjm.2023.137389.1535

Table 1- Results of Variance Analysis (ANOVA) of Incubation Time (days) on *Pleurotus florida* Laccase Activity (U $mL^{-1}$)

| Source of Variation | Degrees of freedom | Mean square | p-value |
|---|---|---|---|
| Time course (days) | 19 | 4.810 | 0.001 |
| Residuals | 60 | 0.02 | |
| Coefficient of consolidation (cv) | 9.4 | | |

Table 2- Results of Variance Analysis (ANOVA) of Inoculation Time and *Rhodotorula mucilaginosa* Cell Concentrations on Laccase Production in *Pleurotus florida*-yeast Cultures

| Source of Variation | Degrees of freedom | Mean square | p-value |
|---|---|---|---|
| Yeast inoculation time | 3 | 30.57 | 0.001 |
| Yeast cell concentration | 2 | 35.13 | 0.001 |
| Yeast inoculation time × yeast cell concentrations | 6 | 4.49 | 0.001 |
| Residuals | 36 | 0.03 | |
| Coefficient of consolidation | 19.25 | | |

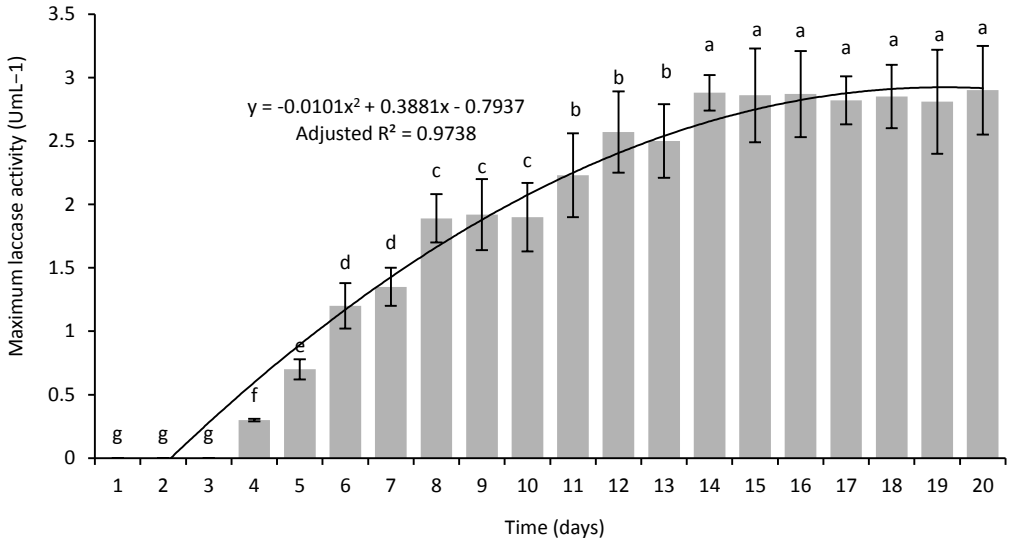


Fig. 1- Time course (days) of laccase activity (U $mL^{-1}$) of *Pleurotus florida* cultivated in potato dextrose broth medium. The columns with the same letter are not significantly different ($P < 0.05$)

The significant influence of inoculation time (Fig. 2A) and the yeast cell concentration (Fig. 2B) of *R. mucilaginosa*, as a single factor, and their interactions were observed in the co-culture interaction of *P. florida* and yeast. A day after the cultivation of *P. florida* with *R. mucilaginosa*, the laccase by *P. florida* on the 14th day was significantly suppressed (Fig. 2A). The limited laccase activities of *P. florida* were almost observed for yeast inoculation after 1 day at all yeast cell concentrations (Fig. 2C). Inoculation of yeast 5 days after *P. florida* cultivation led to obtaining higher laccase activity than other days of cultivation. Maximum laccase activity was determined in *P. florida* culture which was inoculated by yeast after 5 days at the yeast cell concentration of $10^5$ CFU $mL^{-1}$, with 9.2 ±0.52 U $mL^{-1}$ of laccase production on day 14 of *P. florida* cultivation (Fig. 2C). However, an increase in laccase activity was detected after 8 days of *P. florida* cultivation, the increase was not as high as inoculation of the *P. florida* culture after 5 days (Fig. 2A). These results propose that the predominance of *P. florida* in co-culture was involved in enhancing the laccase activity.

http://dx.doi.org/10.22108/bjm.2023.137389.1535

Analysis of variance revealed that the impact of treatments was significant on the laccase production by *P. florida* ($P \leq 0.05$, Table 2). According to the yeast cell concentration of *R. mucilaginosa*, the laccase production in submerged co-cultivation was significantly divided into three levels (Fig. 2B). When the yeast cell concentration in *P. florida* culture was adjusted to $10^5$, and the increasing of laccase production was observed on day 14 of cultivation. In contrast, when the yeast cell concentration was adjusted to $10^3$ CFU $mL^{-1}$, the laccase production of *P. florida* significantly decreased, as compared to $10^5$ or $10^7$ CFU $mL^{-1}$ of yeast cell concentrations (Fig. 2B). The highest amount of laccase production was concerned with $10^5$ CFU $mL^{-1}$ of *R. mucilaginosa* at the 5-day cultures of *P. florida* (Fig. 2C).

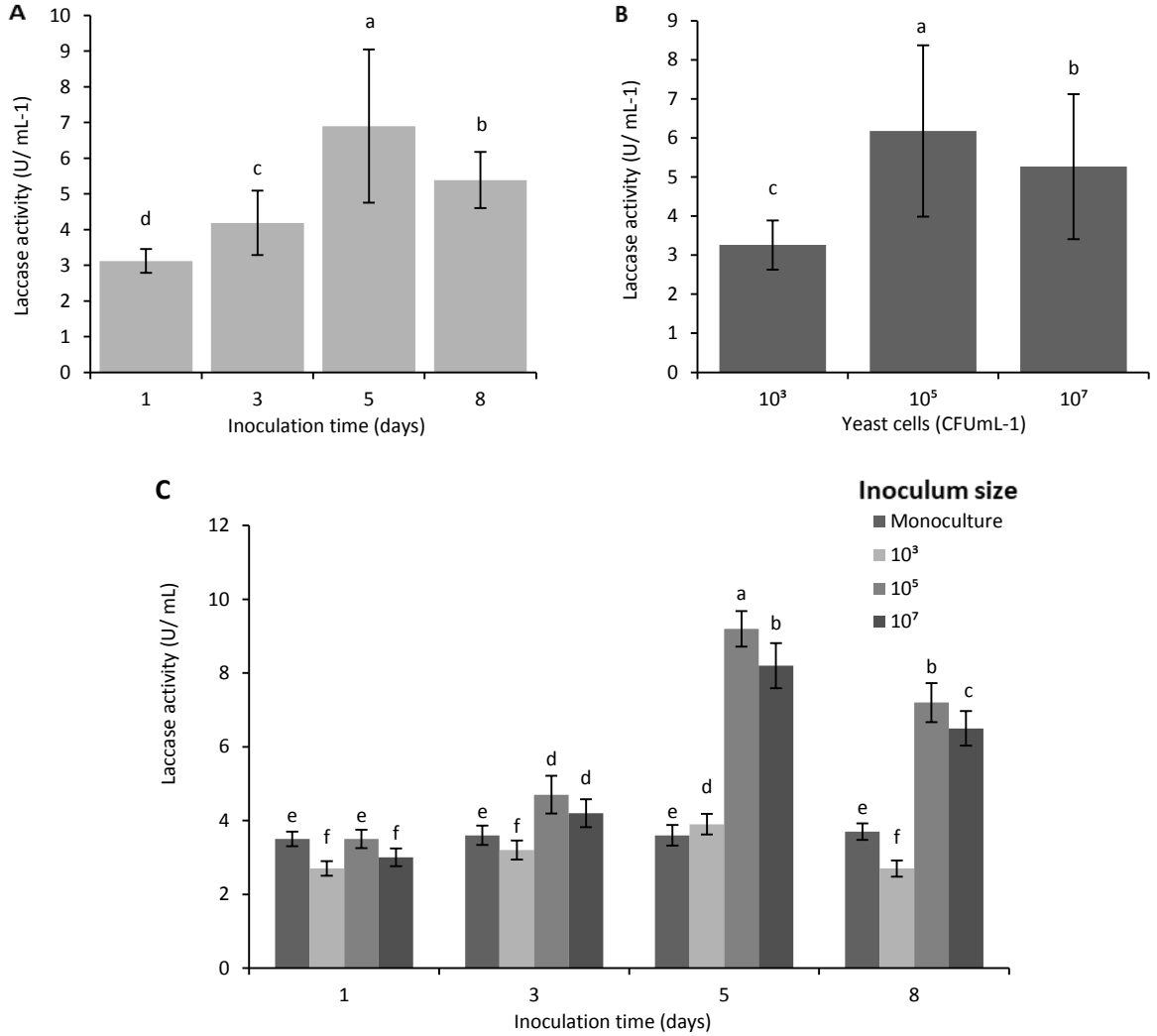


Fig. 2- Mean comparisons of single-factor (a and b) and interaction (c) effects on laccase enhancement on the 14th day in *Pleurotus florida* cultures as affected by the infection time (dates after *P. florida* cultivation, A) and yeast cell concentrations (B) of *Rhodotorula mucilaginosa*. The columns with the same letter are not significantly different ($P < 0.05$)

Analysis of variance revealed that laccase enhancement in *Pleurotus florida* cultures by *Rhodotorula mucilaginosa* sterilized cells at different temperatures was significant ($P \leq 0.05$, Table 3). As shown in Fig. 3, the addition of autoclaved (121 ˚C) *R.*

*mucilaginosa* cells to *P. florida* culture did not significantly enhance laccase production, although yeast cells under the lower temperature (70 ˚C) had a stimulatory effect on laccase activity. This suggested that the live cells of *R. mucilaginosa* were necessary for enhancing laccase production in co-culture and their stimulatory compounds were temperature-sensitive.

Table 3- Results of Variance Analysis of Laccase Enhancement in *Pleurotus florida* Cultures as Affected by *Rhodotorula mucilaginosa* Sterilized Cells at Different Temperatures

| Source of Variation | Degrees of freedom | Mean square | p-value |
|---|---|---|---|
| Cultivation time (days) | 7 | 7.32 | 0.001 |
| Temperature | 2 | 57.07 | 0.001 |
| Cultivation time (days) × Temperature | 14 | 0.43 | 0.05 |
| Residuals | 48 | 0.23 | |
| Coefficient of consolidation | 18.44 | | |

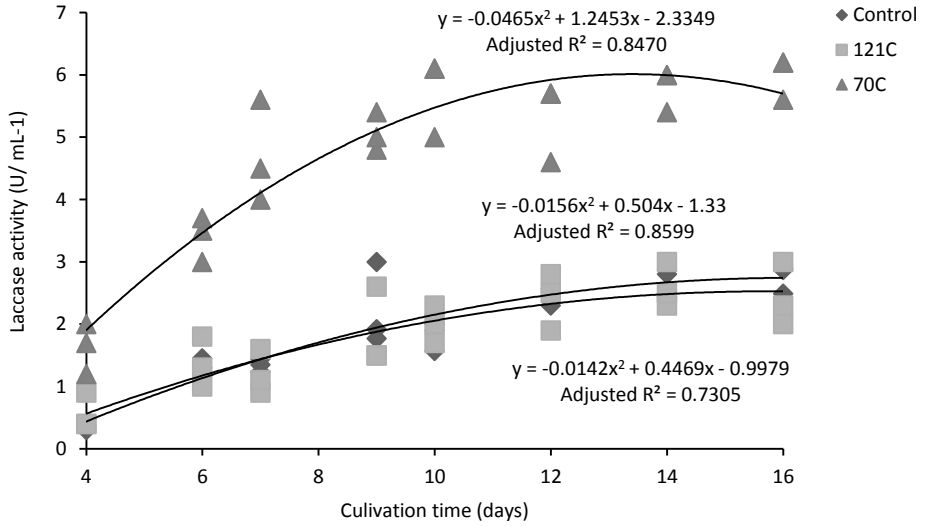


Fig. 3- The effect of sterilized *Rhodotorula mucilaginosa* at different temperatures (121 and 70 ˚C) on laccase production in *Pleurotus florida* cultures. Three percent (v/v) of sterilized cells were added to the *P. florida* cultures

## Discussion and Conclusion

Laccases have a leading position among the commercially produced industrial enzymes in the global market. Therefore, the extent of enzyme production is important for industrial and environmental applications. Microbial enzymes are widely used as cost-efficient and eco-friendly alternatives for chemical processing in different industries and bioremediation. Therefore, the global demand for microbial enzymes is drastically increasing. The incorporation of several nutritional and inductive substances can increase microbial laccase production (9, 20). Therefore, various safe and low-cost methods to enhance laccase production were determined by some researchers. The results of this study indicated the improvement of laccase activity during *P. florida-R. mucilaginosa* interaction, yielding up to 4.5 times activity as compared with control (*P. florida* alone). The interaction of white-rot fungi with bacteria or other fungi for enhancing laccase production has already been investigated by several authors. Enhancement of white-rot fungi laccase production was strongly influenced by co-culturing of *Funalia floccose* with *Penicillium commune* (21); *Lentinula edodes* with *Trichoderma* strains (22, 23) *Pleurotus eryngii* var. *ferulae* with several yeasts (16); *Pleurotus ostreatus* with

http://dx.doi.org/10.22108/bjm.2023.137389.1535

*Bacillus subtilis* (24), *Trichoderma* spp. (24, 25, 26) or *Penicillium* spp. (24); *Trametes* sp. with *Lentinus crinitus* (27), *Paecilomyces carneus* (28), *Sporidiobolus pararoseus* (29), *Trichoderma* spp. (30, 14) or *Penicillium* spp. (24) and *Trychophyton rubrum* with *Trichoderma* spp. (31).

Our results proposed the consideration of the effect of yeast cell concentration and time on *the production* of *laccase* in co-culture, which indicated the importance of the growth balance of the *P. florida* against *R. mucilaginosa*. This indicates enough competitive dominance of *P. florida* in co-culture is needed for the enhancement of laccase production.

Our results showed the higher laccase activities of *P. florida* culture when inoculated with *R. mucilaginosa* in the 5-day culture than those of the 8-day culture, and the inoculation of *P. florida* cultures after 1 day of cultivation suppressed the laccase production. Likewise, Jung (31) showed the higher laccase production of *T. rubrum* when culture inoculation occurred with *T. longibrachiatum* after 5 days of cultivation. The best time for a significant laccase production of *Trametes maxima* with *P. carneus* was also suggested in the 3-day culture of *T. maxima* by Cupul et al. (28).

The results of our experiment showed the $10^5$ CFU $mL^{-1}$ of *R. mucilaginosa* for enhancing the laccase activity by *P. florida*. Cupul et al. (28) emphasized the importance of the number of mycelial disks of *P. carneus* for enhancing the *T. maxima* laccase production in *T. maxima- Paecilomyces carneus* co-cultivation. Velázquez-Cedeno et al. (14) also showed the relatively low spore concentration in the interaction of *P. ostreatus* with *T. longibrachiatum* to colonize liquid or solid medium. These results are in agreement with Jung (31) on the effect of the spore concentration of *T. longibrachiatum* on laccase production in cultures of *T. rubrum*.

These results indicated that laccase production was increased mainly by the live cells of microbial inducers as supported by Baldrian (24), who reported no laccase induction by the addition of sterilized *T. harzianum* culture in *Pleurotus ostreatus* cultures. Also, the results of Jung (14) showed more enhancement of laccase production of the live mycelium of *T. longibrachiatum* than its culture extract or autoclaved mycelium by *T. rubrum* in co-culture. On the other hand, Mata et al. (32) proposed a significantly increased in laccase production of *Plurotus pulmonarius* cultures by inoculation of lytic enzymes of the *Trichoderma* sp. Also, Savoie and Mata (33, 34) suggested that laccase production of *L. edodes* was affected by *Trichoderma* cell wall degrading enzymes. The results are in agreement with Zhang et al. (30) on some thermal-resistant stimulators of *Trichoderma* sp. that can induce laccase production of *Trametes* sp. The results of Guo et al. (16) determine the thermal-sensitive compounds from yeasts that act strongly to induce laccase activity of *P. eryngii* var. *ferulae*. Considering limited work on the enhancement of yeasts on the laccase production of white-rot fungi, our results also showed the presence of temperature-sensitive stimulatory compounds in yeast cells.

The present study explains a partial analysis of the co-culture on the laccase activity in *P. florida*. In the present study, the yeast, *R. mucilaginosa*, effectively enhanced the laccase production in a co-cultivation with *P. florida*. The responses of the laccase production could be affected by the inoculation time (after *P. florida* cultivation) and yeast cell concentration of *R. mucilaginosa*. The interactions need the live stimulator cells and the improvement of laccase production did not occur with yeast-autoclaved cells.

http://dx.doi.org/10.22108/bjm.2023.137389.1535

## Conflict of Interest

The authors declare that there is no conflict of interest.

## Acknowledgment

We appreciate the financial support of this study funded by the Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

## References

(1) Lassouane F., Aït-Amar H., Amrani S., Rodriguez-Couto S. A promising laccase immobilization approach for bisphenol A removal from aqueous solutions. *Bioresour Technology* 2019; 271: 360–7. Doi: 10.1016/j.biortech.2018.09.129.

(2) Shraddha S., Shekher R., Sehgal S., Kamthania M., Kumar A. Laccase: Microbial sources, production, purification, and potential biotechnological applications. *Enzyme Research* 2011; (3): 217861. Doi: 10.4061/2011/217861.

(3) Jeon JR., Chang YS. Laccase-mediated oxidation of small organics: Bifunctional roles for versatile applications. *Trends in Biotechnology* 2013; 31 (6): 335-41. Doi: 10.1016/j.tibtech. 2013.04.002.

(4) Enayatizamir N., Liu J., Wang L., Lin X., Fu P. Coupling laccase production from *Trametes pubescence* with heavy metal removal for economic waste water treatment. *Journal of Water Process Engineering* 2020; 37: 101357. Doi: 10.1016/j.jwpe.2020.101357.

(5) Brijwani K., Rigdon A., Vadlani PV. Fungal laccases: Production, function, and applications in food processing. *Enzyme Research* 2010; (5): 149748. Doi: 10.4061/2010/149748.

(6) Klonowska A., Le Petit J., Tron, T. Enhancement of minor laccases production in the basidiomycete *Marasmius quercophilus* C30. *FEMS Microbiology Letters* 2001; 200 (1): 25-30. Doi: 10.1016/S0378-1097(01)00192-6.

(7) Yadav B. Induction of laccase in fungus, *Cyathus stercoreus* using some aromatic inducers. *Journal of Applied and Natural Science* 2018; 10 (1): 445-7. Doi: 10.31018/jans.v10i1.1648.

(8) Hou H., Zhou J., Wang J., Du C., Yan B. Enhancement of laccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye. *Process Biochemistry* 2004; 39 (11): 1415-9. Doi: 10.1016/s0032-9592(03)00267-x.

(9) Durán-Sequeda D., Suspes D., Maestre E., Alfaro M., Perez G., Ramírez L., Pisabarro AG., Sierra R. Effect of nutritional factors and copper on the regulation of laccase enzyme production in *Pleurotus ostreatus*. *Journal of Fungi* 2022; 8 (1): 7. Doi: 10.3390/jof8010007.

(10) Lomascolo A., Record E., Herpoe-Gimbert I., Delattre M., Robert JL., Georis J., Dauvrin T., Sigoillot JC., Asther M. Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer. *Journal of Applied Microbiology* 2003; 94 (4): 618-24. Doi: 10.1046/j.1365-2672.2003.01879.x.

(11) Wang F., Hu JH., Guo C., Liu CZ. Enhanced laccase production by *Trametes versicolor* using corn steep liquor as both nitrogen source and inducer. *Bioresour Technology* 2014; 166: 602-5. Doi: 10.1016/j.biortech.2014.05.068.

(12) Li P., Wang HL., Liu GS., Li X., Yao JM. The effect of carbon source succession on laccase activity in the co-culture process of *Ganoderma lucidum* and a yeast. *Enzyme and Microbial Technology* 2010; 48 (1): 1-6. Doi: 10.1016/j.enzmictec.2010.07.005.

(13) Hatvani N., Kredics L., Antal Z., Mecs I. Changes in activity of extracellular enzymes in dual cultures of *Lentinula edodes* and mycoparasitic *Trichoderma* strains. *Journal of Applied Microbiology* 2002; 92 (3): 415-23. Doi: 10.1046/j.1365-2672.2002.01542.x.

(14) Velazquez-Cedeno M., Farnet AM., Billette C., Mata G., Savoie JM. Interspecific interactions with *Trichoderma longibrachiatum* induce *Pleurotus ostreatus* defence reactions based on the production of laccase isozymes. *Biotechnology Letters* 2007; 29 (10): 1583-90. Doi: 10.1007/s10529-007-9445-z.

(15) Dong YC., Wang W., Hu ZC., Fu ML., Chen QH. 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 and Biosystems Engineering* 2012; 35: 751-60. Doi: 10.1007/s00449-011-0655-3.

(16) Guo C., Zhao L., Wang F., Lu J., Ding Z., Shi G. β-Carotene from yeasts enhances laccase production of *Pleurotus eryngii* var. *ferulae* in co-culture. *Frontier Microbiolpgy* 2017; 8: 1101. Doi: 10.3389/fmicb.2017.01101.

(17) Razavi SE., Sanei SJ., Taheri AH. Optimization of nutritional factors and copper on lactase production by *Pleurotus florida*. *Microbial Metabolites and Technology* 2021; 4 (1): 39-48. DOI: 10.22104/ARMMT.2022.5881.1077.

(18) 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 and Microbial Technology* 2009; 45 (2): 146-9. Doi: 10.1016/j.enzmictec.2009.04.003.

(19) Galhaup C., Wagner H., Hinterstoisser B., Haltrich D. Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*. *Enzyme and Microbial Technology* 2002; 30 (4): 529-36. Doi: 10.1016/S0141-0229(01)00522-1.

(20) Periasamy R., Palvannan, T. Optimization of laccase production by *Pleurotus ostreatus* IMI 395545 using the Taguchi DOE methodology. *Journal of Basic Microbiology* 2010; 50 (6): 548-56. Doi: 10.1002/jobm.201000095.

(21) Rodríguez RD., Heredia G., Siles JA., Jurado M., Saparrat MC., García-Romera I., Sampedro I. Enhancing laccase production by white-rot fungus *Funalia floccosa* LPSC 232 in co-culture with *Penicillium commune* GHAIE86. *Folia Microbiology (Praha)* 2019; 64 (1): 91-9. Doi: 10.1007/s12223-018-0635-y.

(22) Savoie JM., Mata G., Mamoun M. Variability in brown line formation and extracellular laccase production during interaction between white-rot basidiomycetes and *Trichoderma harzianum* biotype Th2. *Mycologia* 2001; 93 (2): 243-8. Doi: 10.2307/3761644.

(23) Savoie JM., Mata G., Billette C. Extracellular laccase production during hyphal interactions between *Trichoderma* sp. and shiitake, *Lentinula edodes*. *Applied Microbiology and Biotechnology* 1998; 49: 589-93. Doi: 10.1007/s002530051218.

(24) Baldrian P. Increase of laccase activity during interspecific interactions of white-rot fungi. *FEMS Microbiology Ecology* 2004; 50 (3): 245-53. Doi: 10.1016/j.femsec.2004.07.005.

(25) Flores C., Casasanero R., Trejo-Hernandez MR., Galindo E., Serrano-Carreon L. Production of laccases by *Pleurotus ostreatus* in submerged fermentation in co-culture with *Trichoderma viride*. *Journal of Applied Microbiology* 2010; 108 (3): 810-7. Doi: 10.1111/j.1365-2672.2009.04493.x.

(26) Flores C., Vidal C., Trejo-Hernandez MR., Galindo E., Serrano-Carreon L. Selection of *Trichoderma* strains capable of increasing laccase production by *Pleurotus ostreatus* and *Agaricus bisporus* in dual cultures. *Journal of Applied Microbiology* 2009; 106 (1): 249-57. Doi: 10.1111/j.1365-2672.2008.03998.x.

(27) Avelino KV., Halabura MI., Marim RA., Araujo NL., Nunes MG., Silva DL., Colauto GA., Colauto NB., Valle JS. Co-culture of white rot fungi enhance laccase activity and its dye decolorization capacity. *Research, Society and Development* 2020; 9 (11): e88191110643. Doi: 10.33448/rsd-v9i11.10643.

(28) Cupul WC., Abarca GH., Carrera DM., Vázquez RR. Enhancement of ligninolytic enzyme activities in a *Trametes maxima-Paecilomyces carneus* co-culture: Key factors revealed after screening using a Plackett-Burman experimental design. *Eletronic Journal of Biotechnology* 2014; 17 (3): 114-21. Doi: 10.1016/j.ejbt.2014.04.007.

(29) Zhang J., Ke W., Chen H. Enhancing laccase production by white-rot fungus *trametes hirsuta* SSM-3 in co-culture with yeast *Sporidiobolus pararoseus* SSM-8. *Preparative Biochemistry and Biotechnology* 2020; 50 (1): 10-7. Doi: 10.1080/10826068.2019. 1655764.

(30) Zhang H., Hong YZ., Xiao YZ., Yuan J., Tu XM., Zhang XQ. Efficient production of laccases by *Trametes* sp. AH28-2 in co-cultivation with a *Trichoderma* strain. *Applied Microbiology and Biotechnology* 2006; 73 (1): 89-94. Doi: 10.1007/s00253-006-0430-6.

(31) Jung HC. Enhancement of laccase production from wood-rotting fungus by co-culture with *Trichoderma longibrachiatum*. *Journal of the Korean Wood Science and Technology* 2019; 47 (2): 210-20. Doi: 10.5658/WOOD.2019.47.2.210.

(32) Mata G., Murrieta-Hernandez DM., Iglesias-Andreu, LG. Changes in lignocellulolytic enzyme activities in six *Pleurotus* spp. strains cultivated on coffee pulp in confrontation with *Trichoderma* spp. *World Journal of Microbiology and Biotechnology* 2005; 21 (2): 143-50. Doi: 10.1007/s11274-004-3041-3.

(33) Savoie JM., Mata G. The antagonistic action of *Trichoderma* sp. hyphae to *Lentinula edodes* hyphae changes lignocellulolytic activities during cultivation in wheat straw. *World Journal of Microbiology and Biotechnology* 1999; 15 (3): 369-73. Doi: 10.1023/A:1008979701853.

(34) Savoie JM., Mata G. *Trichoderma harzianum* metabolites pre-adapt mushrooms to Trichoderma aggressivum antagonism. *Mycologia* 2003; 95 (2): 191-9. Doi: 10.2307/3762030.