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Screening of ligninolytic enzymes in 21 macrofungi species from the Noun division in the Western Highlands of Cameroon

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Abstract

Fungi are among organisms producing molecules with potential biotechnological applications like ligninolytic enzymes capable of breaking down recalcitrant molecules. Few species especially from tropical Africa have been screened for this purpose. In this study, ligninolytic enzymes of fresh sporophore extracts of 21 wild mushrooms including saprotrophic, ectomycorrhizal and termite associated species from savannah and forest galleries of the Noun Division in the West Region of Cameroon were screened. The specific activities of the enzymes were determined spectrophotometrically using their corresponding substrates ABTS, methylene blue and guaiacol for laccase, lignin peroxidase and manganese peroxidase, respectively. All species produced crude proteins with *Tylopilus* sp. having the highest content. Enzymatic activities were observed in all species; 18 produced the three enzymes while 3 produced two enzymes. Laccase was found in all species and had the highest specific activity (0.0220 – 17.5994 U/mg) followed by manganese peroxidase (0.0005 – 0.1992 U/mg) and lignin peroxidase (0.0005 – 0.0278 U/mg). The highest ligninolytic enzyme content was found in *Coriolopsis polyzona* with specific activities of laccase, manganese peroxidase and lignin peroxidase of 17.5994 U/mg, 0.1336 U/mg and 0.0007 U/mg, respectively. This screening should be extended to more species and enzymes of species presenting best activities characterized for their potential application in various biotechnological fields such as bioremediation, reagent development in medical and environmental fields.

1. Introduction

Ligninolytic enzymes catalyze the breakdown of the complex and recalcitrant polymer lignin (a component of the lignocellulosic material of plants). They are also capable of degrading substances with structure similar to that of lignin such as phenolic and non-phenolic aromatic compounds, various environmental pollutants including polycyclic aromatic hydrocarbons, synthetic dyes, pesticides, polychlorinated biphenyls and many other xenobiotics due to their extracellular and non- specific nature [1]. This ability allows them to be used in various biotechnological areas such as bioremediation, biofuel, food, agriculture, paper and pulp, textile finishing, denim stone washing, cosmetics, biosensors etc. [2]. The three main ligninolytic enzymes are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) [3]. Lignin peroxidases (diaryl propane: oxygen,

hydrogen peroxide oxidoreductase, EC 1.11.1.14) are heme-containing peroxidases that catalyze the hydrogen peroxide dependent oxidation of non-phenolic aromatic compounds into cation radicals which are further decomposed chemically. It preferentially cleaves the Cα-Cβ lignin molecule but is also capable of ring opening and other reactions [4]. Manganese peroxidases (Mn(II): hydrogen peroxide oxidoreductase, E.C. 1.11.1.13) are also heme containing peroxidases that oxidize phenolic compounds to phenoxy radical by oxidation of Mn(II) to Mn(III) ion in the presence of hydrogen peroxide (H_2O_2) as a cofactor [4-5]. The Mn(III) ion obtained is chelated by organic acids such as oxalate or malate in nature. Once chelated, it oxidizes phenolic lignin subunits to phenoxy radicals that degrade spontaneously [4-5]. Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are blue multicopper oxidases that catalyze the oxidation of an array of aromatic substrates concomitantly with the reduction of molecular oxygen to water [6].

Ligninolytic enzymes sources include bacteria, plants and fungi [7]. However, fungi, in particular white rot fungus, are the most potent sources of the enzymes [3]. Macrofungi just like other fungi are heterotrophic and obtain the nutrients necessary for their growth through three different ways: saprophytism, parasitism (pathosystic symbiosis) and mutualistic symbiosis [8]. Saprobe mushrooms (wood decomposing and litter decomposing fungi) secret these enzymes when growing in their natural habitat to degrade the lignin in the substrate on which they grow [9]. Also, the *Termitomyces* mushrooms species which live in a mutualistic association with termites provide the ligninolytic enzymes needed by their termite's partners to breakdown lignin in wood while the termites in turn provide a suitable environment for the mushroom growth [10]. In addition, widespread occurrence of laccases and peroxidases genes have been recently demonstrated in ectomycorrhizal (ECM) mushrooms even though their real ligninolytic potential in symbiosis with plant roots and the impact of ectomycorrhizal formation on the secretion of these enzymes by the host plant and the ectomycobiont is almost unknown [7].

African mushrooms and more specifically those from Cameroon have not yet been extensively documented and the potentials of known species are still poorly studied [11-12-13]. The search of biological activity from these organisms in Cameroon in terms of nutritional, pharmacological or biotechnological properties is yet to be discovered. Therefore, the main objective of this study was to screen the three main ligninolytic enzymes in the fresh fruit body extracts of some mushroom species collected from the Noun Division in the West region of Cameroon.

2. Materials & Methods

2.1. Sample collection and identification

The specimens were collected from the Noun divisions in the West region of Cameroon. Collections were done in March 2016 in the savannahs and forest galleries of Mamarom, the forest Reserve in Foumban subdivision and Mamevoue in Koutaba subdivision on different substrates (Table 1). Twenty-one mushroom species of different trophic group were collected and identified morphologically using literature on tropical African mushrooms including [14-15]. The specimens were cleaned with a knife and washed with tap water to discard contaminated soil. Then, they were packaged in plastic bags and stored at -20°C in order to preserve the ligninolytic enzyme activities.

2.2. Crude enzyme extract preparation

The crude enzyme was extracted according to a modified method of Erden et al. and Teixeira et al. [16-17]. The mushroom fruiting bodies were blended with 0.02 M sodium phosphate buffer pH 6.0 (10 mL of buffer for 1g of mushroom). The mixture obtained was centrifuged (+4°C, 4500 rpm for 15 minutes) after 1h of incubation in an ice bath. The resulting supernatant was stored in aliquots of 5 mL at –20°C and used subsequently for the determination of total protein content and ligninolytic enzymes activities.

Table1: Mushrooms species collected and used in the study

Mushroom species and taxonomic group	Substrate	Ecosystem	Mode of life
Afroboletus luteolus (Heinem.) Pegler & T.W.K. Young	Soil	Forest	ECM
Amanita rubescens Pers.	Soil	Forest	ECM
Lactifluus persicinus Delgat & De Crop,	Soil	*Uapaca* forest	ECM
*Lactifluus gymnocarpus (*R. Heim ex Singer) Verbeken	Soil	*Uapaca* forest	ECM
Lactifluus rubroviolascens (R. Heim) Verbeken	Soil	*Uapaca* forest	ECM
Termitomyces aurantiacus (R. Heim) R. Heim	Soil	Savannah	TA
Termitomyces clypeatus R. Heim	Soil	Savannah	TA
Termitomyces letestui (Pat.) R. Heim	Soil	Savannah	TA
Termitomyces mboudaiena Mossebo	Soil	Savannah	TA
Termitomyces schimperi (Pat.) R. Heim	Soil	Savannah	TA
Tylopilus sp.	Soil	Savannah	ECM
Xerocomus sp.	Soil	Savannah	ECM
Coriolopsis polyzona (Mont.) Ryvarden	DW	Forest	Saprotrophic
Echinochaete brachypora (Mont.) Ryvarden	DW	Forest	Saprotrophic
Ganoderma lucidum (Curtis) P. Karst.	DW	Forest	Saprotrophic
Lentinus cladopus Lév.	DW	Forest	Saprotrophic
Lentinus squarrosulus Mont.	DW	Forest	Saprotrophic
Neonothopanus hygrophanus(Mont.) De Kesel & Degreef	DW	Forest	Saprotrophic
Pleurotus pulmonarius (Fr.) Quél.	DW	Farmland/ Savannah	Saprotrophic
Polyporus tenuiculus (P. Beauv.) Fr. (P. Beauv.) Fr.	DW	Forest	Saprotrophic
Schizophyllum commune Fr. ex Fr.	DW	Forest	Saprotrophic

DW: Dead Wood, ECM: Ecto-mycorrhizal, TA: Termite Associated

2.3. Determination of the total crude protein content of the extracts

Protein concentration was determined according to the Bradford method using bovine serum albumin (BSA) as standard [18]. For calibration, different quantities of BSA ranging from 0 to 20 µg were used. The test was prepared by the addition of adequate volume of 0.02 M phosphate buffer pH 6.0; 100 µL of the fresh fruit body extract and appropriate volume of Bradford reagent. The mixture obtained was homogenized, incubated at room temperature for 10 minutes and the optical density was read at 595 nm against the blank. The linearity relationship between absorbance and amount of protein was plotted and the standard curve was used to determine the concentration of protein.

2.4. Determination of ligninolytic enzymes activity

Lignin peroxidase (LiP) activity was determined by the demethylation of methylene blue as substrate into Azure C in the presence of H_2O_2 (inducer). The assay solution contained 1000 µL of 50 mM sodium potassium tartrate buffer (pH 4.0), 100 µL of 0.1 mM H_2O_2, 100 µL of 32 mM methylene blue and 50 µL of the crude enzyme extract. The mixture was homogenized and then incubated at 37°C for 1hour. After incubation, the decreased in absorbance was measured at 650 nm using a spectrophotometer. A control tube in which the crude enzyme extract was replaced with 50 µL of distilled water was included. One unit of LiP activity was defined as the amount of enzyme that decolourises 1.0 µmole of methylene blue per minute following Bholay et al. [19].

Manganese peroxidase (MnP) activity was determined by Mn^{2+}-dependent oxidation of guaiacol as substrate. The reaction mixture contained 100 µL of 0.4 mM H_2O_2, 1 mL of 50 mM sodium tartarate (pH 4.5) buffer permanently incubated at 37°C, 100 µL of 1 mM $MnSO_4.H_2O$, 200 µL of 1 mM guaiacol and 100 µL of the crude extract. The mixture was homogenized and then absorbance increase

recorded at 465 nm every 5 minutes for 25 minutes. The control tube contained all the reagents as the other tubes except that the extract was replaced by 100 µL of distilled water. Also, one unit of MnP activity was defined as the amount of enzyme that oxidized 1.0 µmole of guaiacol per minute according to Shantaveera & Ramalingappa [20].
Laccase (Lac.) activity was assayed in a reaction mixture containing 2 mL, 50 mM phosphate buffer (pH 4.0; 37°C), 50 µL, 1 mM of substrate (ABTS) and 10 µL of the crude extract. The mixture was homogenized and the increase in absorbance recorded at 420 nm. A control tube with 10 µL of distilled water replacing the crude extract was included. Following Narkede [21], one unit of Lac activity was defined as the amount of enzyme that oxidized 1.0 µmole of ABTS per minute.

2.5. Data analysis
The total crude protein contents were determined by means of the standard protein curve equation. Protein quantity in mg/g of fresh mushroom and the specific activity of each ligninolytic enzyme expressed in U/mg (µmole product released.min^{-1}.mg^{-1} protein) were calculated using the formulae stated below. Mean and standard deviation (SD) of the results based on two independent experiments carried out in duplicates were calculated.

$$\text{Protein quantity in mg/g of fresh mushroom} = \frac{PQ \times TV}{AV \times m} \quad (1)$$

Where: PQ = protein quantity (µg); TV=total volume of reaction mixture (mL);
AV=assay volume (mL); m = mass of fresh mushroom (g)

$$SAenz = \frac{\Delta OD}{T \times PC \times AV} \quad (2)$$

Where: SAenz = Specific activity of enzyme; ΔOD = Change in optical density; T = Time (min);
PC = Protein concentration (µg/mL); AV = Assay volume (mL).

3. Results and Discussion

3.1. Crude protein contents of fresh fruit bodies
All species involved in the study produced proteins with content varying according to species. The highest amount was observed in *Tylopilus* sp. (3.677 ± 0.128 U/mg), followed by *Amanita rubescens* (2.218 ± 0.004 U/mg), *Neonothopanus hygrophanus* (1.771 ± 0.016 U/mg), *Lactifluus rubroviolascens* (1.768 ± 0.007 U/mg) and *Pleurotus pulmonarius* (1.723 ± 0.040 U/mg). *Termitomyces schimperi* and *Polyporus tenuiculus* showed the lowest amount respectively 0.271 ± 0.009 U/mg and 0.434 ± 0.059 U/mg (figure 1).
It is well known that mushroom species produce proteins with concentration varying with species [22]. The present study exhibits the same trend with the majority of ECMs having the highest crude protein contents, followed by wood-inhabiting saprotrophic species and termites associated species. This could be due to the fact that some ECMs species have saprotrophic potential that allows them to produce or obtain proteins as ECMs or saprotrophic [23]. However, ligninolytic activities seem not to be the major function of the proteins contents of these fungi as their laccases and peroxidases activities are higher rather in wood-inhabiting saprotrophic species.

3.2. Ligninolytic enzyme in fresh fruit body extracts
Concerning the Ligninolytic enzyme activities, all the 21 mushroom species screened presented an activity of at least one of the three enzymes searched (table 2). LiP was absent only in *Xerocomus* sp. and found in 20 species. Its specific activities ranged from 0.0005 ± 0.0004 U/mg in *Lactifluus*

gymnocarpus to 0.0278 ± 0.0017 U/mg in *Lentinus cladopus* (Figure 1). Except in *Termitomyces schimperi* and *Schizophyllum commune*, MnP was found in all other species involved in the study with highest activity found in *Lactifluus gymnocarpus* (0.1992 ± 0.0158 U/mg) and lowest activity in *Lentinus squarrosulus* (0.0005 ± 0.0000 U/mg).

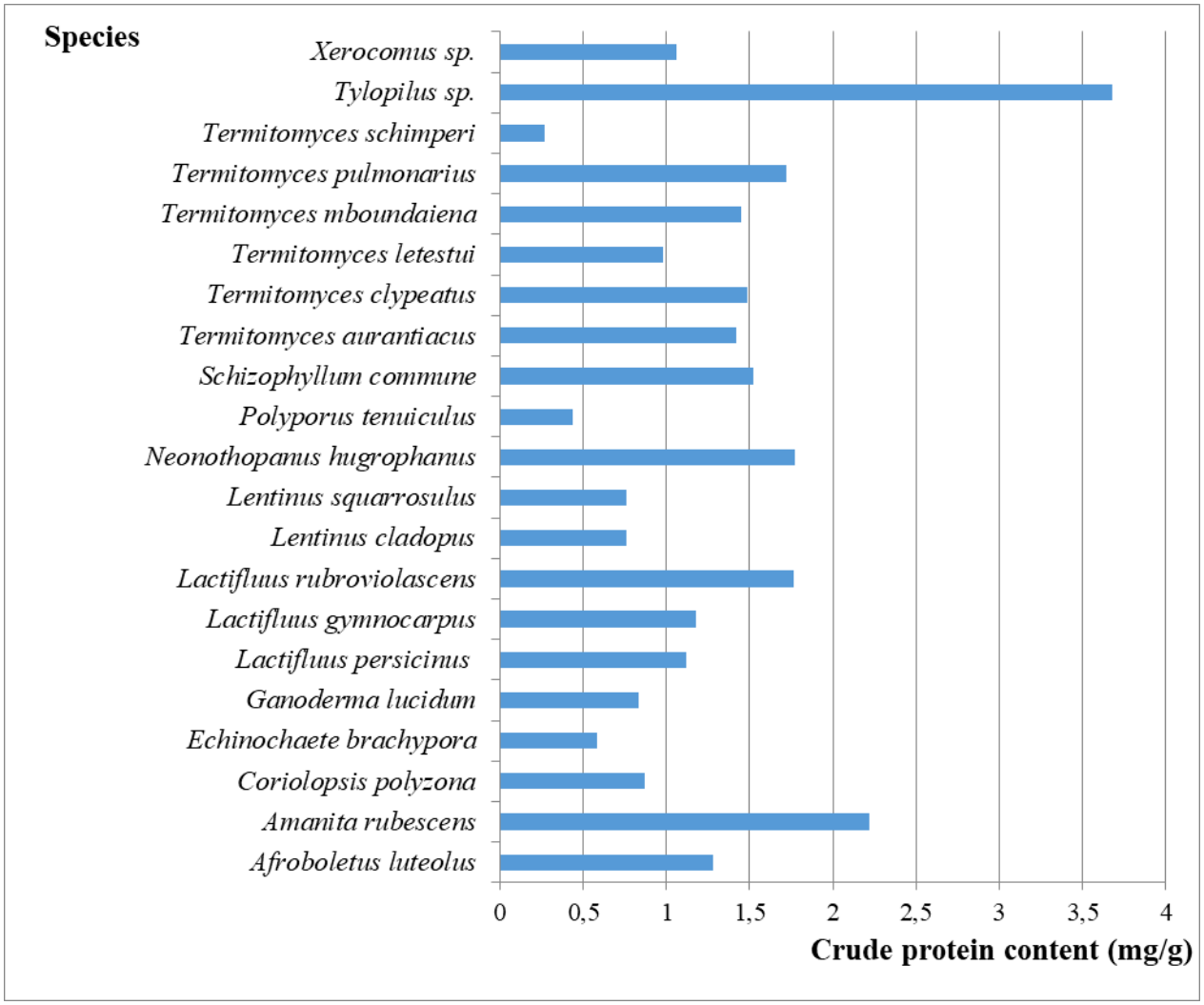

Figure 1: Fresh fruit body soluble crude protein content of mushroom species

Laccase activity found in all species was highest among the three enzymes in the extracts. The specific activity of this enzyme ranged from 0.0220 ± 0.0000 U/mg in *Xerocomus* sp. to 17.5994 ± 0.1406 U/mg in *Coriolopsis polyzona*. Its activity was followed by that of MnP that varied from 0.0005 ± 0.0000 U/mg to 0.1992 ± 0.0158 U/mg respectively in *Lentinus squarrosulus* and *Lactifluus gymnocarpus*. Finally, LiP presented the lowest activities from 0.0005 ± 0.0001 U/mg in *Lactifluus gymnocarpus* to 0.0278 ± 0.0017 U/mg in *Lentinus cladopus* (Table 2).

Globally, among the studied species, 18 species including termite associated mushrooms (*Termitomyces letestui*, *Termitomyces mboudaiena*, *Termitomyces clypeatus*, *Termitomyces aurantiacus)*, wood inhabiting saprobe species (*Pleurotus pulmonarius*, *Lentinus squarrosulus*, *Lentinus cladopus*, *Echinochaete brachypora*, *Neonothopanus hygrophanus*, *Coriolopsis polyzona*, *Polyporus tenuiculus* and *Ganoderma lucidum*) and ectomycorrhizal species (*Afroboletus luteolus, Amanita rubescens*, *Lactifluus gymnocarpus*, *Lactifluus persicinus*, *gymnocarpus rubroviolascens*,

Tylopilus sp. and *Xerocomus* sp.) presented all the three ligninolytic enzymes activities in their fresh fruiting bodies. *Termitomyces schimperi* and *Schizophyllum commune* presented only laccase and LiP activities, while *Xerocomus* sp. showed the activities of both laccase and MnP. The highest ligninolytic enzyme content was obtained in *Coriolopsis polyzona*.

Table 2: Overall ligninolytic enzymes specific activities of species

Species	LiP (U/mg)	MnP (U/mg)	Laccase (U/mg)
Afroboletus luteolus	0.0007 ± 0.0000	0.1389 ± 0.0097	0.1719 ± 0.0000
Amanita rubescens	0.0007 ± 0.0000	0.0802 ± 0.0000	0.2006 ± 0.0000
Coriolopsis polyzona	0.0053 ± 0.0025	0.1336 ± 0.0001	17.5994± 0.1406
Echinochaete brachypora	0.0049 ± 0.0008	0.0295 ± 0.0139	2.3026 ± 0.2170
Ganoderma lucidum	0.0034 ± 0.0016	0.0011 ± 0.0001	0.4339 ± 0.3068
Lactifluus persicinus	0.0012 ± 0.0001	0.0925 ± 0.0037	12.3124 ± 0.1169
Lactifluus gymnocarpus	0.0005 ± 0.0001	0.1992 ± 0.0158	7.7630 ± 0.0989
Lactifluus rubroviolascens	0.0006 ± 0.0003	0.0038 ± 0.0000	0.1211 ± 0.0054
Lentinus cladopus	0.0278 ± 0.0017	0.0272 ± 0.0077	1.6972 ± 0.4800
Lentinus squarrosulus	0.0072 ± 0.0023	0.0005 ± 0.0000	0.3944 ± 0.1859
Neonothopanus hygrophanus	0.0036 ± 0.0001	0.0108 ± 0.0000	1.3548 ± 0.4790
Pleurotus pulmonarius	0.0021 ± 0.0004	0.0577 ± 0.0000	1.9752 ± 0.1214
Polyporus tenuiculus	0.0047 ± 0.0029	0.0457 ± 0.0043	6.2771 ± 0.8070
Schizophyllum commune	0.0007 ± 0.0004	-0.0023 ± 0.0033	0.1442 ± 0.0000
Termitomyces aurantiacus	0.0030 ± 0.0027	0.1275 ± 0.0193	14.3111 ± 2.8444
Termitomyces clypeatus	0.0024 ± 0.0003	0.0015 ± 0.0007	1.9669 ± 0.2140
Termitomyces letestui	0.0014 ± 0.0001	0.0027 ± 0.0002	2.2349 ± 0.0000
Termitomyces mboudaiena	0.0014 ± 0.0003	0.0010 ± 0.0001	1.0299 ± 0.1120
Termitomyces schimperi	0.0029 ± 0.0013	-0.0531 ± 0.0500	0.0737 ± 0.0001
Tylopilus sp.	0.0020 ± 0.0003	0.0008 ± 0.0006	0.1251 ± 0.0000
Xerocomus sp.	- 0.0068 ±0.0037	0.0529 ± 0.0075	0.0220 ± 0.0000

The negative sign signifies the absence of a ligninolytic enzyme activity in the fruit body extracts of the species.

In this study, and for the first time, the fresh fruit body extracts of 21 mushroom species from the Noun Division in the western Region of Cameroon were screened to determine the activities of laccases, manganese peroxidase and lignin peroxidase which are the three principal ligninolytic enzymes involve in lignocellulose degradation produced by Basidiomycetes. The result shows that all species including saprotrophic and symbiotic species present the activities of at least two of the above mentioned enzymes with specific variation in the intensity of their activities. However, the intensity of activities obtained may not represent the real enzymatic capacity of the species as it has been demonstrated that the ligninolytic activity of some enzyme varies with the age of fruit bodies [24]. Laccases, MnP and LiP activities were also detected in ectomycorrhizal and saprotrophic fruiting bodies of other mushroom species from Andhra Pradesh in India [4] and from Bornova Izmir in Turkey [16]. The detection of ligninolytic enzymes in the fruit bodies of all the studied species can be explained by the fact that, in addition to breaking down lignocellulose, these enzymes especially

laccases are also involved in other physiological activities of mushroom such as growth and morphogenesis [25-26-27]; thus these enzymes could be found in the large majority of fungi with intensity of their activities varying according to their specific mode of life.
Using spot tests on mycelia growing on MEA media of some Cameroonian wood rotten fungi, [11-28] noticed high enzymatic activities of laccases and peroxidases in *Pleurotus pulmonarius, Lentinus squarrosulus, Lentinus cladopus, Echinochaete brachypora, Nothopanus hygrophanus* and *Coriolopsis polyzona*. They considered these species as white rotten fungi as well as *Schizophyllum commune* where only the presence of laccases was noticed. The present work using their fruit bodies support the results of the above authors. Also, the highest ligninolytic enzyme specific activities were showed by *C. polyzona* which was also found among species having high enzymatic activities by the above authors. *C. polyzona* is a well known saprotrophic species that grows on dead wood, exhibiting activities of the three ligninolytic enzymes for their used in the breakdown of lignin and other wood constituencies into simpler substances necessary for its growth and metabolic need. Its bioremediation capacity has been effectively demonstrated on some waste like olive oil mill waste waters using mycelia culture [29].
Concerning the termite associated species, apart from *Termitomyces schimperi*, this is the one of the first time that ligninolytic enzyme activities are screened from the fresh fruit bodies of species involved in the present work. This demonstrates that species of this genus produce the studied enzymes especially laccases and can be classified in white rot group of fungi. Working on *T. schimperi,* Haileka [30] also detected laccases in its crude extract. The laccase activities in *Termitomyces* spp. are explained by the fact that species of this genus form a mutualistic symbiosis with termites. The latter mashes and swallow different parts of plants and then, rejects them without digesting in the form of a nest where *Termitomyces* spores find suitable conditions for their optimum growth and development into fruiting bodies producing ligninolytic enzymes to digest the plant constituents nest. This is later consumed again by termites to obtain energy in return [10].
This study demonstrated the production of ligninolytic enzymes in ectomycorrhizal species. These enzymes, mainly laccases have already been reported in ectomycorrhizal taxa [31-32] and there is increasing evidence that these enzymes contribute not only to the functioning of the symbiosis but also to the mobilization of nutrients [23]. In addition, widespread occurrence of laccases and peroxidases genes has been demonstrated in ectomycorrhizal mushroom species [8].

Conclusion

According to this study, ligninolytic enzymes are produced by both saprotrophic and ectomycorrhizal mushroom species with each species having different levels of lignin peroxidase, manganese peroxidase and laccase activities. Laccase seems to be the dominant enzymes in the studied species with *Coriolopsis polyzona* the species with the highest ligninolytic enzyme activities. It is interesting to extend the screening of these enzymatic activities to other species of the area, characterized the enzymes especially those that presented the best activities like *C. polyzona* and applied them in various biotechnological fields. Therefore, they could be potential sources of commercial enzymes for industrial use in bio-bleaching of pulp, decolourization of textile dyes and bioremediation of polluted environment among other uses.

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References

1. G.Y S. Mtui, Lignocellulolytic enzymes from tropical fungi: types, substrates and applications, *Scientific Research and Essays*, 7 (2012) 1544–1555. DOI: 10.1111/j.1469-8137.2005.01401.x
2. M. Asgher, Q. Yasmeen, M. N. Sheikh and H. Nawaz, Optimization of ligninolytic enzyme production through response surface methodology, *Biores*, 8(1) (2013) 944–968. DOI: 10.15376/biores.8.1.944-968
3. J. Placido, S. Capareda, Ligninolytic enzymes: a biotechnological alternative for bioethanol production, *Bioresour Bioproces*, 2 (2015) 1–12. DOI: 10.15376/biores.8.1.944-968
4. D. Seshikala, C.M.A. Singara, Collection and screening of basidiomycetes for better lignin degraders, *Int. J. Life Sci. Biotechnol & Pharma Res*, 1(4) (2012) 203–211. http://www.ijlbpr.com/index.php?m=content&c=index&a=show&catid=117&id=474
5. A. Koumar, R. Chandra, Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment, *Heliyon,* 6(2) (2020). https://doi.org/10.1016/j.heliyon.2020.e03170
6. A.B. Vantamuri, B.B. Kaliwal, Purification and characterization of laccase from *Marasmius* species BBKAV79 and effective decolorization of selected textile dyes, *Biotech*, 6 (2016) 1–10. https://doi.10.1007/s13205-016-0504-9
7. S.C. Prakram, Role of various bacterial enzymes in complete depolymerization of lignin: A review, *Biocatalysis and Agricultural Biotechnology*, 23 (2020) 101498. https://doi.org/10.1016/j.bcab.2020.101498
8. A. Anastasi, V. Tigini, G.C. Varese, The bioremediation potential of different eco-physiological groups of fungi. In: EM Goltapeh et al., editors. Fungi as Bioremediators, Soil Biology. 32nd ed. Berlin Heidelberg, Germany: Springer-Verlag,. https://doi.10.1007/978-3-642-33811-3_2
9. C.V. Petre, T. Balaes, C. Tanase, Lignicolous basidiomycetes as valuable biotechnological agents. Memoirs of the Scientific Sections of the Romanian Academy, 37 (2014): 37–62.
10. D.D. Tibukha, *Termitomyces* species from Tanzania, their Cultural properties and unequalled Basidiospores, *J. Biol. Life Sci*., 3(1) (2012) 140–159. http://dx.doi.org/10.5296/jbls.v3i1.1723
11. D.C. Mossebo, A.L. Njouonkou, R. Courtecuisse, A. Amougou, Enzymatic activities and decay characteristics in some wood-rotting basidiomycetes from Cameroon and determination of time dependent activity of syringaldazine in spot tests**,** *Cryptog, Mycol.*, 28(2) (2007) 107–121**.**
12. O. Mahamat, A.-L. Njouonkou, C. Tume, A.M. Abamukong, A. Kamanyi, Assessment of antimicrobial and immunomodulatory activities of termite associated fungi, *Termitomyces clypeatus* R. Heim (Lyophyllaceae, Basidiomycota), *Clinical Phytoscience*, 4 (2018). https://doi.org/10.1186/s40816-018-0089-4.
13. O. Mahamat, C. Tume, A.-L. Njouonkou, J. Amadoh, N. Nfor, A. Kamanyi, Screening of the immunomodulatory and antibacterial activity of *Termitomyces letestui* (Pat.) Heim (Lyophyllaceae), an edible mushroom from Cameroon, *Journal of Basic and Clinical Physiology and Pharmacology* 29(6) (2018) 645–650. DOI: 10.1515/jbcpp-2017-0189
14. C. Douanla-Meli, Fungi of Cameroon Ecological diversity with emphasis on the taxonomy of non-gilled Hymenomycetes from the Mbalmayo forest reserve, Bibliotheca Mycologica, ISBN: 978-3-443-59104-5 (2007).
15. D.N. Pegler, A preliminary agaric flora of East Africa, Kew Bulletin, ISBN: 13 9780112411017 (1977).
16. E. Erden, M.C. Ucar, T. Gezer, N.K. Pazarlioglu' Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of Remazole marine blue, *Brazilian J. Microbiol*, 40(2) (2009) 346–353. DOI: 10.1590/S1517-838220090002000026

17. R.S.S. Teixeira, P.M. Pereira, V.S. Ferreira-Leitão, Extraction and Application of laccases from Shimeji Mushrooms (*Pleurotus ostreatus*) Residues in Decolourisation of Reactive Dyes and a Comparative Study Using Commercial Laccase from *Aspergillus oryzae*, *J. Enzym Res*, (2010) 1–8. https://doi.org/10.4061/2010/905896
18. M.M. Bradford, A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of Protein-Dye Binding, *Anal Biochem.,* 72 (1976) 248–254. https://doi.org/10.1016/0003-2697(76)90527-3
19. A.D. Bholay, B.V. Borkhataria, P.U. Jadhav, K.S. Palekar, M.V. Dhalkari, P.M. Nalawade, Bacterial lignin peroxidase: A tool for Biobleaching and biodegradation of industrial effluents, *Univers*. *J. Environ. Res. Technol,* 2(1) (2012) 58–64.
20. S.H.M. Shantaveera, Ramalingappa, Lignolytic enzymes production from selected mushrooms, *Int. J. App. Sci. Biotechnol*, 3(2) (2015) 308–313. https://doi.org/10.3126/ijasbt.v3i2.12732
21. M. Narkhede, R. Mahajan, K. Narkhede, Ligninolytic enzyme production and Remazol brilliant blue r (RBBR) decolorization by a newly isolated white rot fungus: Basidiomycota spp. 1-168, *Int. J. Pharm. Biol. Sci*., 4(1) (2013) 220–228.
22. E. Boa,Wild edible fungi A global overview of their use and importance to people, *Non-Wood Forest Products* 17, FAO, ISBN 92-5-105157-7 (2004).
23. K. Pritsch, J. Garbaye, Enzyme secretion by ECM fungi and exploitation of mineral nutrients from soil organic matter, *Ann. Forest. Sci.,* 68 (2011) 25–32. https://doi.10.1007/s13595-010-0004-8
24. P. Bhupathi, A.S. Krishnamoorthy, S. Uthandi, Profiling of morphogenesis related enzymes of milky mushroom *Calocybe indica* (P & C), *J Pharmacog Phytochem*, 6(5) (2017) 2537–2543.
25. C.D. Marr, D.W. Grund, K.A. Harrisson, The taxonomic potential of laccase and tyrosinase spot tests. *Mycologia*, 78(2) (1986) 169–184. https://doi.org/10.1080/00275514.1986.12025228
26. A.-L. Njouonkou, D.C. Mossebo, Etude du genre *Pleurotus* (Fr.) Kummer du Cameroun: morphologie, caractères culturaux et enzymes extracellulaires, *J Biol. Biochem. Sci.*, 20 (2012) 10–20.
27. Z. Deng, A. Xia, Q. Liao, X. Zhu, Y. Huang, Q. Fu Laccase pretreatment of wheat straw: effects of the physicochemical characteristics and the kinetics of enzymatic hydrolysis, *Biotechnol Biofuels,* 12 (159) (2019) https://doi.org/10.1186/s13068-019-1499-3
28. F. Peláez, M.J. Martinez, A.T. Martinez, Screening of 68 species of basidiomycetes for enzymes involved in lignin degradation, *Mycol. Res*, 99(1) (1995) 37–42. https://doi.org/10.1016/S0953-7562(09)80313-4
29. A. Jaouani, M.G. Tabka, M.J. Penninckx, Lignin modifying enzymes of *Coriolopsis polyzona* and their role in olive oil mill wastewaters decolourisation, *Chemosphere*, 62(9) (2006) 1421–1430. https://doi.org/10.1016/j.chemosphere.2005.05.040
30. V.L. Haileka, Screening, isolation and characterization of laccase enzymes from Namibian *Termitomyces schimperi* and *Kalaharituber pfeilii.* Master, The University of Namibia, Windhoek, Namibia (2015).
31. R. Agerer, M. Schloter, C. Hahn, Fungal enzymatic activity in fruit bodies, *Nova Hedwigia*, 71(3) (2000) 315–336.
32. P.E. Courty, K. Pritsch, M. Schloter, A. Hartmann, J. Garbaye, Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests, *New Phytologist*, 167(1) (2005) 309–319. https://doi.org/10.1111/j.1469-8137.2005.01401.x