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Assessment of extracellular enzymes in mycelial culture of some fungi from the Western highlands of Cameroon

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Abstract: The aim of this study was to screen mushrooms species from the Western Highlands of Cameroon for their ability to produce some extracellular enzymes. Firstly, the mycelia of 18 saprotrophic macrofungi from the Western Highlands of Cameroon were grown on malt extract broth for 30 days at 25°C under static conditions. Then, the activity of the enzyme's laccase, manganese peroxidase, lignin peroxidase, amylase, cellulase and invertase were determined spectrophotometrically in the liquid media using their corresponding substrates. The tested macrofungi produced varied levels of the 6 enzymes. All 18 tested species produced invertase, laccase and amylase with *Pleurotus djamor* having the highest activities of these enzymes respectively 8,753±0,084U/mg, 433,785±2,947U/mg and 10.718±2.947U/mg. Ten species produced lignin peroxidase and 8 species produced cellulase with highest activities respectively 0,357±0,084U/mg in *Pleurotus djamor* and 0.397±0,037U/mg in *Microporus xanthopus*. Lastly 6 species produced manganese peroxidase (MnP) with highest activity in *Ganoderma applanatum* (0,116±0,001U/mg). This is the first report of the production of these enzymes in liquid culture media in Cameroon and central Africa in general and globally, the activity of some enzymes were reported for the first time in some species that is *Microporus xanthopus*, *Lenzites acuta*, *Lentinus critinus* for amylase, invertase, laccase; *Trametes elegans* for LiP and MnP, *Microporus xanthopus*, *Lenzites acuta*, *Coriolopsis polyzona* for cellulase irrespective of the culture conditions. This study therefore presents these species as sources of these biotechnologically important enzymes.

1. Introduction

Fungi are integral part of different ecosystems involved in processes such as nutrient cycling and decomposition of organic matter (Díaz-Godínez *et al.,* 2016). Macrofungi like members of Agaricomycotina (Basidiomycota) comprise various physiological groups including white rot, brown rot and soft rot species with some being edible and/or medicinal (Erden *et al.,* 2009), (Krupodorova *et al.,* 2014). White rot fungi are able to degrade all the major components (lignin, cellulose and hemi cellulose) of wood (Sánchez-Corzo *et al.*,2021), while brown rot fungi have the capacity to breakdown

cellulose and hemi-cellulose from the wood leaving behind only brown lignin residue whereas the soft rot fungi degrade cellulose and hemi-cellulose, but only little lignin (Andlar *et al.,* 2018). These degradations are being achieved through the secretion of various extracellular ligninolytic, cellulolytic, proteolytic and lipolytic enzymes (Dhevagi *et al.* 2021) among which we have lignin peroxidase, manganese peroxidase, laccase, cellulase, xylanase, β-glucosidase, xylanase, pectinase, invertase, amylases and so on (Elkhateeb et al., 2022). Laccases (Lac) (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are enzymes that contain copper in their catalytic site, which are known as multicopper oxidases, and are responsible for catalysing the oxidation of several organic and inorganic substrates, with simultaneous reduction of oxygen to water (Titi *et al.*,2023; Chevreuil *et al.*,2022; Jalal *et al.*, 2020). Manganese peroxidases (MnP) (EC 1.11.1.13) is a heme-containing peroxide-dependent glycoprotein that oxidizes the one-electron donor Mn^{2+} to Mn^{3+}, which in turn can oxidize a large number of phenolic substrates (Ezeh *et al.,* 2019), (Chang *et al.,* 2021). Lignin peroxidase, LiP, (EC 1.11.1.14, 1, 2-bis (3,4-dimethoxyphenyl propane-1,3-diol: hydrogen-peroxide oxido reductase) catalyse the hydrogen peroxide dependent depolymerisation of lignin and other phenol, non-phenolic lignin model compounds (Agrawal *et al.,* 2017). Cellulase catalyses the hydrolysis of 1, 4 β-D glycosidic linkages in cellulose (Debnath *et al.,* 2019). Invertase (EC.3.2.1.26) belongs to glycoside hydrolases that catalyses the hydrolysis of sucrose (table sugar) into two monosaccharides i.e. glucose and fructose (Manoochehri *et al.,* 2020). Amylases act on α –1,4 and α-1,6 glycosidic linkages present in starch and glycogen. They catalyse the hydrolysis of starch molecules into sugars such as maltose and dextrin, as well as smaller polymers made up of glucose units (Mondal *et al.*,2022).

These enzymes are characterized by broad specificity in degradation/mineralization of different complex substrates such as wood, paper, animal feeds, pesticides, biofuels, and hydrocarbons (Asemoloye *et al.,* 2021). This accounts for their wide range of biotechnological applications (Kango *et al.,* 2019); (EL-Gendi *et al.,* 2021); (Ghorai *et al.,* 2022) such as the detoxification of industrial effluent, mostly from, textile and petrochemical industries, bleaching and delignification processes in the paper and pulp industries, removing the phenolic compounds from the beer and wine in the food industry (Makela *et al.* 2016); (Kurkina *et al.* 2021); (EL-Gendi *et al.* 2021). These applications in biotechnology have stimulated the investigation of their production with the purpose of selecting promising enzyme producers and increasing of their yield (Kalmıs *et al.,* 2008) through varying culture conditions like pH, temperature, carbon, nitrogen sources, and metal ions (Manawadi *et al.,* 2019). Therefore, various fungal organisms must initially be screened for these biotechnologically important enzymes activities and their quantitative enzyme production determined (El Ouadi *et al.,* 2015; Kim, 2021). Similarly, it is more important to elucidate different enzyme activities in wild organisms for applied and environmental sciences (Shartaveera and Ramalingappa, 2015). Existing studies on macrofungi in Africa, specifically Cameroon in terms of extracellular, enzymes activities are few and still limited to qualitative detection of the enzymes using spot tests (Mossebo *et al.*, 2007; Njouonkou *et al.,* 2012) and quantitative determination of the enzyme's activities from the fresh fruiting bodies (Ematou *et al.*, 2020).

Therefore, this study focuses on the production of the aforementioned enzymes by mycelia culture of 18 mushrooms collected from the forest and savannah of the Western Highlands of Cameroon. The bioprospecting of fungal organisms isolated from tropical environments is a topic of great interest in biotechnological processes such as biofertilizers, biofuels, bioremediation, biological control, and food processing, detergents, textile, and pharmaceutical industries (Peraza-Jimenez *et al.,* 2022).

2. Methodology

2.1 Mushroom sample collection, identification and mycelial preparation

The fresh fruit bodies of 18 saprotrophic mushroom species (**Table 1**) were collected from various ecosystems of savannah Western Highlands agro-ecological zone of Cameroon mainly in the Noun division (West Region) and Bamenda town (North-West Region). Samples were identified morphological using literature on tropical African mushrooms including Douanla-Meli. (2007), Niemelä *et al*., (2021) and Ryvarden *et al*., (2022). The website of index fungorum (https://www.indexfungorum.org/names/Names.asp) and mycobank (https://www.mycobank.org/Basic%20names%20search) were used to determine the current names of species. For conveniences concerning some species, we used the most used name and, the current name in the above websites are put in bracket in table 1. After collection, clean context or hymenium of fresh fruit body was aseptically inoculated on malt extract agar (MEA) medium prepared prior to the field trip in small transparent bottles (used as test tube) and incubated at room temperature. Back to the laboratory the mycelia obtained were transferred on MEA medium in petri dishes and the plates incubated for 7 days for mushroom mycelium growth. The mycelium collected from the growing edge was transferred into new malt agar plates and incubated further for 7 days. This was repeated 2 to 3 times to get pure isolates which were stored at 4°C.

Mycelia plugs (8 mm in diameter) taken from 7 days old cultures on malt extract agar plates were inoculated in liquid medium malt extract broth (MEB) (2%, 100 ml) prepared in 250 mL flask. The liquid below the mycelium was taken after 30 days of cultivation, the content of the flask filtered through a whatmann No.1 paper and residues discarded. The filtrate was recovered as the crude enzyme extract and used for protein determination and extracellular enzymes assays.

Table 1. Mushroom species studied and enzymes produced

Species	Family	Collection Ecosystem	Enzymes produced
Ganoderma applanatum (Pers.) Pat.	Ganodermataceae	Farmland	Lac, amylase, invertase, MnP
[1]*Ganoderma boninense* Pat. (Syn: *G. orbiforme* (Fr.) Ryvarden)	Ganodermataceae	Farmland	Lac, amylase, invertase, cellulase
Ganoderma lucidum (Fr.) P. Karst.	Ganodermataceae	Farmland	Lac, invertase, amylase, LiP, MnP
Ganoderma resinaceum Boud.	Ganodermataceae	Gallery Forest	Lac, invertase, amylase, LiP
Lentinus crinitus (L.) Fr.	Polyporaceae	Forest plantation of *Eucalyptus*	Lac, invertase, amylase, LiP
Lentinus retinervis Pegler	Polyporaceae	Forest plantation of *Eucalqyptus*	Lac, invertase, amylase
Lentinus sajor-caju (Fr.) Fr.	Polyporaceae	Farmland	Lac, invertase, amylase, LiP, MnP
Lentinus squarrosulus Mont.	Polyporaceae	Farmland	Lac, LiP, cellulase, amylase, invertase
Lenzites acuta Berk. (Syn: *Cellulariella acuta* (Berk.) Zmitr. & Malysheva)	Polyporaceae	Forest plantation of *Eucalyptus*	Lac, amylase, invertase, cellulase

Microporus xanthopus (Fr.) Kuntze	Polyporaceae	Forest plantation of *Eucalyptus*	Lac, amylase, invertase, cellulase
Pleurotus djamor (Rumph. ex Fr.) Boedijn	Pleurotaceae	Farmland	Lac, LiP, MnP, amylase, invertase, cellulase
Pleurotus pulmonarius (Fr.) Quél.	Pleurotaceae	Forest plantation of *Eucalyptus*	Lac, invertase, amylase, MnP
Pleurotus tuber-regium (Fr.) Singer	Pleurotaceae	Farmland	Lac, invertase, amylase, LiP, MnP
Polyporus tenuiculus (P. Beauv.) Fr. (*Favolus tenuiculus* P. Beauv.)	Polyporaceae	Forest plantation of *Eucalyptus*	Lac, LiP, amylase, invertase
Pycnoporus sanguineus (L.) Murrill	Polyporaceae	Forest plantation of *Eucalyptus*	Lac, invertase, amylase, cellulase, MnP
Trametes lactinea (Berk.) Sacc. (*Cubamyces lactineus* (Berk.) Lücking)	Polyporaceae	Farmland	Lac, invertase, amylase
Coriolopsis polyzona (Pers.) Ryvarden (*Trametes polyzona* (Pers.) Justo)	Polyporaceae	Farmland	Lac, LiP, amylase, invertase, cellulose
Trametes elegans	Polyporaceae	Forest plantation of *Eucalyptus*	Lac, LiP, MnP, amylase, invertase

[1]This species is known to live as parasite especially of palm oil tree (Elaeis guineensis) Khoo and Chong, (2023). Our samples were collected at the base of this plan

2.2 Protein determination and enzymes assays

Protein content in crude extracts was determined by the lowry (1951) method using bovine serum albumin (BSA) as a standard.

Laccase was measured according to the method described by Narkhede *et al*., (2013) as modified by Ematou *et al*., (2020) using ABTS (2,2 azino bis ethyl benzothiazoline -6- Sulphonic acid) as substrate. One unit of Lac activity was defined as the amount of enzyme that oxidized 1.0 µmole of ABTS per minute.

Manganese peroxidase was assayed by the method described by Sasidhara and Thirunalasundari, (2014) with some modifications. Briefly, 0.5 mL sodium tartarate buffer (100 mM, pH 5.0) was taken and added with 0.5 mL MnSO4 (100 mM) followed by 0.5 mL guaiacol (100 mM) and 0.1 mL of enzyme culture filtrate and 1 ml of distilled water. The contents were shaken thoroughly and 0.5 mL of H_2O_2 (50 mM) was added and immediately the absorbance was observed at 465 nm for every 20 seconds and enzyme activity was expressed in units/min (U/min).

Lignin peroxidase was assayed in a mixture consisting of 250 mL sodium tartarate (pH 3.0), 2 mM veratryl alcohol, 0.4mM H_2O_2 and 100 µL of enzyme in a total volume of 0.5 mL. The reaction was initiated by addition of H_2O_2 and oxidation of veratryl alcohol to veratralatehyde was determined by an increase in absorbance at 310 nm as described by Purnomo *et al*., (2010) and Rajwart *et al*., (2016). Cellulase and invertase were assayed according to Tellez-Tellez *et al*., (2016) using the substrates carboxymethyl cellulose and sucrose respectively.

Amylase was assayed using starch as substrate dissolved in phosphate buffer pH 5.9 in a reaction mixture containing 2 mL substrate and 50 uL enzyme extract. The reaction was stopped with 0.1M

HCl at 2 minutes' interval for 10 minutes before 100 ul of iodine solution was added. The optical density was recorded at 620 nm (Wanderley *et al*., 2004).

2.3 Data analysis

All experiments were carried out in triplicates and the results are the average of two closest values and are expressed as mean ± standard deviation (SD).

Protein quantity was expressed in mg/g of fresh mycelium while the specific activity of each enzyme in U/mg (micromole of product released. min^{-1} .mg^{-1} protein) The software XLSTAT was used for principal component analysis of variables; mushroom species and enzymes produced.

3. Results and discussion

3.1 Protein determination

All the studied mushroom species in this study produce extracellular crude proteins. The range of protein quantity varies from 133.465± 0.561 mg/g of fresh mycelium in *L. critinus* to 0.252 ± 0.019mg/g of fresh mycelium in *P. djamor* (Figure 1).

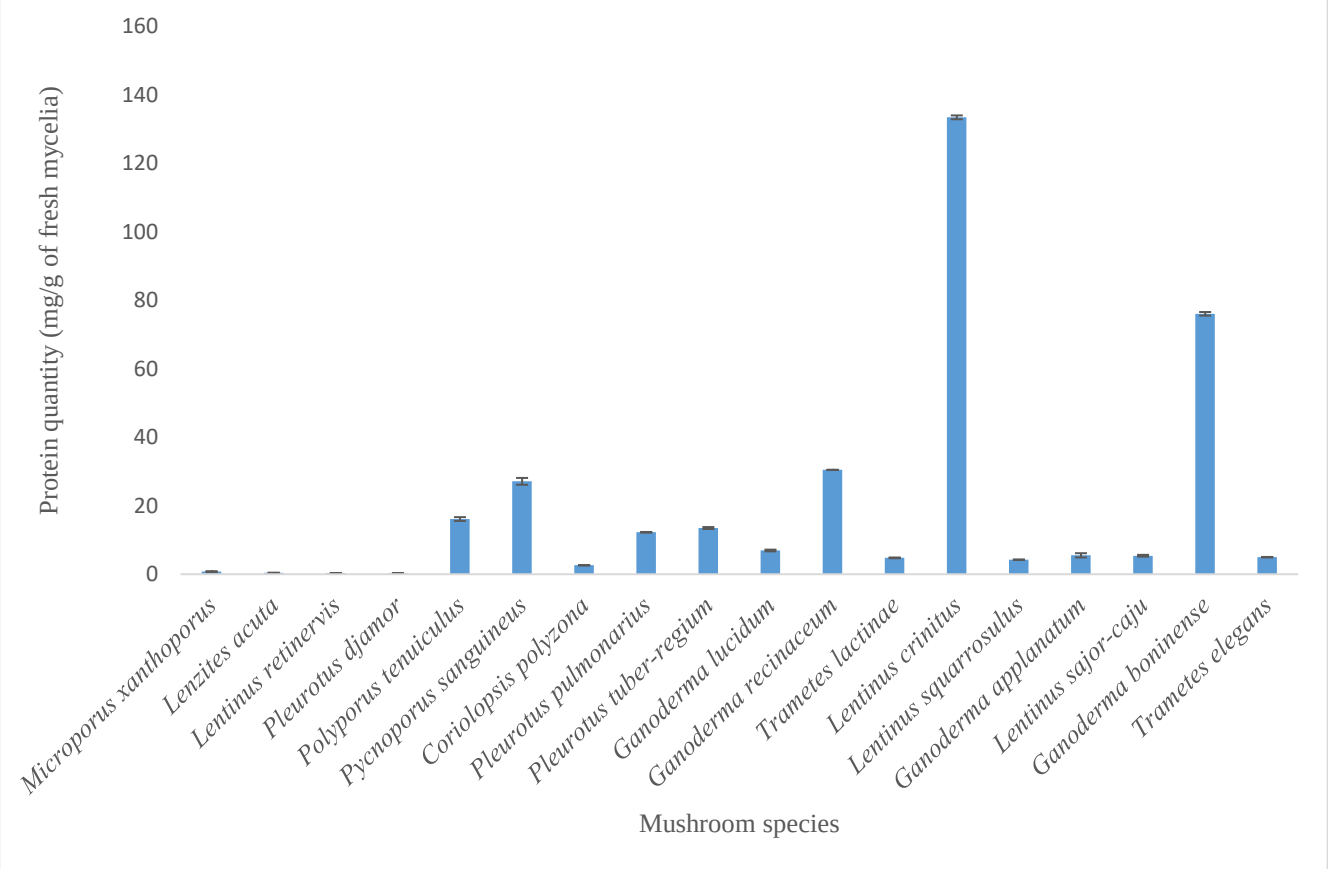

Figure 1. Soluble crude protein content of mushroom specie

3.2 Extracellular enzymes produced and activities

All 18 screened mushroom species produced extracellular enzymes. The highest number of enzymes was obtained with *P. djamor* that produced all the six tested enzymes while the lowest number was observed in *L. retinervis* and *T. lactinae* where only three of the six enzymes were detected. Nine species produced four enzymes while six produced five enzymes (**Table 1**). Among the 18 mushroom species, 17 are saprotrophic (decomposers) while one (*G. boninense*) is a parasite living on dead palm

tree and hence they breakdown the lignocellulosic material (cellulose and lignin) in their substrate (soil, wood) into simpler products which they take up as nutrients by secreting enzymes (Eichlerova and Baldrian, 2020), (Kumla *et al*., 2020), (Nargotra *et al*., 2023). In addition to breaking down lignocellulose, these enzymes especially laccases are also involved in other physiological activities of mushroom such as growth and morphogenesis; 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 (Diaz-Godinez *et al*., 2016).

Amylase was produced by all the tested species. The highest production was observed with *P. djamor* and *L. acuta* with respectively 10.718±2,947 U/mg and 10.090±1.209 U/mg. The lowest specific activity levels were observed in *G. boninense* with 0.434±0.073 U/mg and *P. sanguineus* with 1.184±0.137 U/mg (**Table 2**).

Eight species produced cellulase with their specific activities ranging from 0.039±0.007U/mg in *G. boninensis* to 0.397±0,037U/mg in *M. xanthopus* (**Table 2**). The activity of invertase was observed in the media of all species (Table 2). Here, *P. djamor* yield the highest activities with 8.753±0.084 U/mg while *G. boninensis* (0.138±0.014 U/mg) and *P. sanguineus* (0.161±0.0394U/mg) manifested the lowest activities. Laccase activities were also noticed in culture media of all species involved in the study with variable activities among species (**Table 2**). *P. djamor* had the greatest activity, followed by *M. xanthopus* with respectively 433.785±2.947 U/mg and 38.777±1.048 U/mg. The least activities were found in *L. crinitus* and *G. boninensis* with specific activities of 0.009±0,002 U/mg and 0.056±0.006 U/mg respectively. Ten species produced lignin peroxidase. Their specific activities ranges from 0,011±0,005U/mg in *G. lucidum* to 0,357±0,084U/mg in *P. djamor* (**Table 2**). Finally, only 6 species produced manganese peroxidase (Table 2). The highest specific activity was 0.116 ± 0.001 U/mg with *G. applanatum* and lowest activity was 0.004 ± 0.000 U/mg in *P. pulmonarius*.

As showed on **Table 2**, all the tested strains produced at least 3 enzymes (invertase, amylase and laccase) out of the 6 at different levels. The production of these enzymes by all the tested strains can be explained by the fact that they breakdown the malt on which they were grown into simpler substances which they took up as nutrients necessary for their growth (Kumla *et al*., 2020), (Nargotra *et al*., 2023) and in particular amylase and invertase production by all the 18 species tested is due to the fact that they decompose starch and sucrose (in the malt) into glucose and fructose respectively (Santana de Almeida et al., 2005, Matei *et al*., 2017, Upadhyay *et al*., 2021).

There are some studies on the production of these 6 extracellular enzymes by some of the mushroom species investigated in this study and include; the production of lignocellulosic enzymes (laccase, MnP, LiP, cellulase) by *Pleurotus* spp. when cultivated on different lignocellulosic substrates (Omoanghe *et al*., 2009), production of laccases by several *Pleurotus* strains (*Pleurotus erygii*, *Pleurotus pulmonarius*, *Pleurotus djamor* and *Pleurotus ostreatus*) grown for 12 days at 28°C on malt extract and glucose/yeast media (Nelma *et al*., 2021), the of high Lac, MnP and LiP activities in *Pleurotus djamor* respectively 14.05 × 10^{-6} IU/mL, 19.19 ×10^{-6} IU/mL and 10.64 ×10^{-6}IU/mL grown on potato dextrose broth after 21 days of cultivation (Illuri *et al*., 2021) similar to the present study regardless of the difference in culture conditions.

Nonetheless, culture conditions influence enzyme production in other cases supporting the absence of the production of cellulase by *P. tuber-regium* as mentioned by Fasiku *et al*., (2023). Furthermore, Tellez-Tellez *et al*., 2016, reported the extracellular activities of six hydrolases (proteases, invertase, cellulase, amylases pectinases, xylanases) and laccases produced by three wild mushrooms (*Lentinula boryana*, *Pleurotus djamor* var. roseus and *Pycnoporus* sp.) grown on potato-dextrose agar and wheat straw-dextrose agar and found varied levels of these enzymes in all the strains.

Table 2. Extracellular enzymes activities of mushroom species

Mushroom species	Specific amylase activity (U/mg)	Specific invertase activity(U/mg)	Specific laccase activity (U/mg)	Specific cellulase activity (U/mg)	Specific manganese peroxidase activity (U/mg)	Specific lignin peroxidase activity (U/mg)
Coriolopsis polyzona	2.023±0.431	2.256±0.007	3.035±0.053	0.216±0.007	ND	0.022±0.015
Ganoderma applanatum	3.787±1.604	1.182±0.018	0.775±0.033	ND	0.116±0.001	ND
Ganoderma boninense	0.434±0.073	0.138±0.014	0.056±0.006	0.039±0.007	ND	ND
Ganoderma lucidum	2.249±0.187	0.172±0.005	0.039±0	0.070±0.005	ND	0.011±0.005
Ganoderma recinaceum	2.485±0.800	0.175±0.058	0.020±0.005	ND	ND	0.075±0.004
Lentinus crinitus	1.527±0.062	0.326±0.047	0.009±0.002	ND	ND	0.012±0.009
Lentinus retinervis	5.619±2.207	1.037±0,055	10.379±0.331	ND	ND	
Lentinus sajor-caju	1.360±0.265	0.344±0.131	0.107±0.058	ND	ND	0.015±0.003
Lentinus squarrosulus	2.774±0.030	1.245±0.030	3.988±0.030	0.065±0.006	ND	0.056±0.006
Lenzites acuta	10.090±1.209	0.684±0.048	15.350±0.423	0.342±0.096	ND	ND
Microporus xanthopus	2.844±0.467	0.913±0.542	38.777±1.048	0.397±0.037	ND	ND
Pleurotus djamor	10.718±2.947	8.753±0.084	433.785±2.947	0.089±0.042	0.074±0.021	0.357±0.084
Pleurotus pulmonarius	3.909±1.302	0.184±0.023	0.242±0.009	ND	0.004±0.000	ND
Pleurotus tuber-regium	7.484±1.490	0.558±0.193	1.188±0.018	ND	0.049±0.003	0.026±0.006
Polyporus tenuiculus	2.347±0.387	1.408±0.055	0.136±0.027	ND	ND	0.019±0.005
Pycnoporus sanguineus	1.184±0.137	0.161±0,039	0.097±0.019	0.183±0.031	0.050±0.007	ND
Trametes elegans	3.400±0.156	0.348±0.031	4.544±0.152	ND	0.026±0.009	0.030±0.004
Trametes lactinae	1.780±0.040	0.397±0.035	0.038±0.013	ND	ND	ND

Notes- All values are mean ± SD of duplicates determinations. ND stands for not determined.

Several reports also documented the production of ligninolytic enzymes by *Ganoderma* spp. (Fonseca *et al*., 2010, Torres-Farrada *et al*., 2017). According to Zhou *et al*., (2012), some *Ganoderma* strains can produce only one or two out of the three ligninolytic enzymes depending on media composition and culture conditions (Kumla *et al*., 2020). The same was obtained for the Ganoderma species of this study. The genus *Lentinus* is known to be made up of white rot species that have been reported to produce at least one lignocellulolytic enzymes grown under different cultivation conditions (Tirado-Gonzalez *et al*., 2016). The *Lentinus* species involved in this study (*Lentinus retinervis*, *Lentinus squarrosulus*, *Lentinus crinitus* and *Lentinus sajor-caju*) produced both laccase, lignin peroxidase and

cellulose as lignocellulosic enzymes. In addition, none of the *Lentinus* species produced MnP, however, the production of this enzyme by members of this genus had been reported in other studies on other culture media. For instance; Mossebo *et al*., (2007) detected it on *Lentinus* spp. cultivated on MEA using spot tests and Conceicao *et al*., (2017) noticed it production by *Lentinus crinitus* grown on agro-industrial wastes with high enzyme activity (23.5 U/L). Out of the 3 ligninolytic enzymes (laccase, lignin peroxidase, manganese peroxidase) tested, laccase was produced by all the 18 species with highest specific activities than the peroxidases (LiP and MnP). This can be correlated to the reports of Isroe *et al*., (2011) who postulated that generally laccases are more widely distributed among mushrooms especially the white rot fungi than the peroxidases. Jaouani *et al*., 2006 reported the production of these three ligninolytic enzymes (LiP, MnP, Lac) by *C. polyzona* where the enzymes production was regulated by $Mn^{2+}$and veratryl alcohol whereas in this study the same species produced just two of the ligninolytic enzymes (LiP and Lac).

Principal component analysis was done in order to classify the mushroom species as sources of the enzymes. Amylase and invertase present a positive correlation with *G. applanatum, G.boninense, L.sajor-caju, L.crinitus, G.lentinus, P. tenuiculus, G.retinaceum, T. lactinae and P. sanguineus. On the contrary, laccase presents a positive correlation with C.polyzona, L.squarrosulus, P. pulmonarius, P.djamor, T.elegans, P. tuber-regium, L. retinervis, M. xanthopus* and *L.acuta*. However, the enzymes cellulase, lignin peroxidase and manganese peroxidase do not present any correction with the mushrooms samples (Figure 2). This implies *G. applanatum, G. boninense, L. sajor-caju, L. crinitus, G. lucidum, P. tenuiculus, G. retinaceum, T. lactinae and P. sanguineus are potent sources of amylase and invertase while C.polyzona, L. squarrosulus, P. pulmonarius, P.djamor, T.elegans, P. tuber-regium, L. retinervis, M. xanthopus* and *L. acuta* are potent sources of laccase.

From **Figure 2**. above, it emerges that amylase and invertase present a positive correlation with the species *G. applanatum, G. boninense, L.sajor-caju, L. crinitus, G. lucidum, P.tenuiculus, G. recinaceum, T.lactinae and P. sanguineus whereas, laccase presents a positive correlation with the species C. polyzona, L. sajor-caju, P. pulmonarius, P,djamor, T. elegans, P. tuber-regium, L. retinervis, M. xanthopus* and *L. acuta*. However, the enzymes cellulase, lignin peroxidase and manganese peroxidase doesn't present any correlation with the species.

To the best of our knowledge, up to date, most studies on the production of enzymes by macrofungi in tropical Africa, especially the central African area concerned ligninolytic enzymes including laccase, peroxidases and tyrosinase (Mossebo, 2002, Mossebo *et al*.,2007, Njouonkou 2011, Ematou *et al*., 2020, Tsigain *et al*., 2022). This study investigated for the first time the production of all the studied enzymes in liquid culture media in Cameroon and central Africa in general. Furthermore, it gives the first report on the production of amylase, cellulase and invertase by macrofungi of this area irrespective of culture conditions such as media type, time of cultivation which influences extracellular enzymes (Bentil *et al*., 2018).

It also clearly differentiated the production of LiP and MnP that in the study of Mossebo (2002), Mossebo *et al*. (2007), Njouonkou (2011), and Tsigain *et al*., (2022) were noticed just as peroxidases. Globally, some of the investigated mushrooms in this study are reported as species producing the extracellular enzymes search in this study for the first time: *Microporus xanthoporus, Lenzites acuta* and *Lentinus critinus* for amylase, invertase and laccase; *Trametes elegans* for LiP and MnP; *Microporus xanthopus, Lenzites acuta* and *Coriolopsis polyzona* for cellulase irrespective of culture conditions such as media type and time of cultivation.These results showed that the local mushrooms are a potential source of enzyme producers and the activity values might be increased by modifying the culture conditions of the mushrooms.

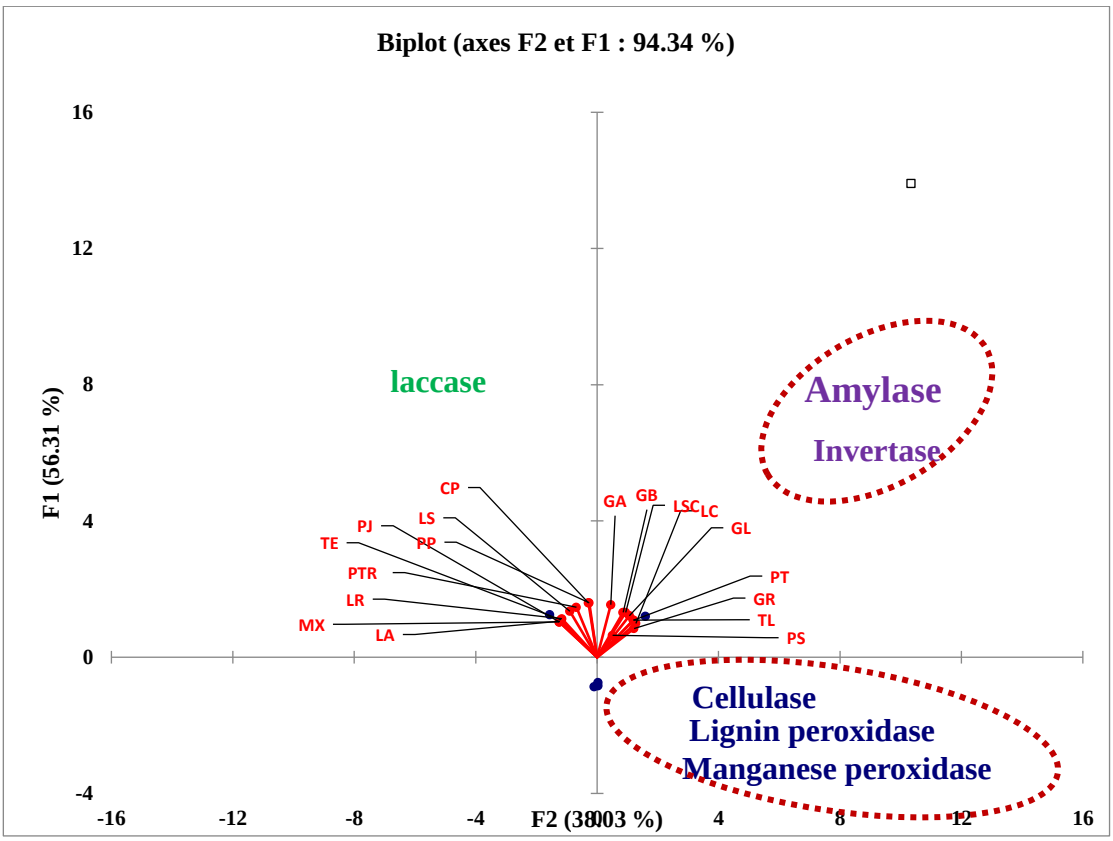

Figure 2. Principal components analysis of the mushroom species

MX=*Microporus xanthopus*, LA=*Lenzites acuta*, LR=*Lentinus retinervis*, PJ=*Pleurotus djamor*, PT=*Polyporus tenuiculus*, PS= *Pycnoporus sanguineus*, CP=*Coriolopsis polyzona*, PP=*Pleurotus pulmonarius*, PTR=*Pleurotus tuber-regium*, GL= *Ganoderma lucidum*, GR=*Ganoderma recinaceus*, TL=*Trametes lactinae*, LC=*Lentinus critinus*, LS=*Lentinus squarrosulus*, GA=*Ganoderma applanatum*, LSC=*Lentinus sajor-caju*, GB=*Ganoderma boninensis*, TE=*Trametes elegans*

F1 represents 56.03% of the species while F2 represents 38.01% of them.

Conclusion

The present study highlights the potential of tropical African mushroom species especially from the Western Highlands Agro-ecological zone of Cameroon to produce the 6 industrial and biotechnological important enzymes namely: amylase, invertase, cellulase, laccase, manganese peroxidase and lignin peroxidase in liquid culture media. Varied specific enzymes activities levels were obtained for the 18 mushroom species with *P. djamor* having the highest activity and being the only species to have produced all 6 enzymes investigated. Extensive researches on these extracellular enzymes under varied culture conditions such as media type, days of cultivation, pH of culture media, presence of inducers are required in order to determine the best conditions for higher enzymes production by the studied species and therefore apply it in the large scale production for their biotechnological applications such as bio-bleaching of pulp, decolourization of textile dyes, bioremediation of polluted environment amongst other uses.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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