LIGNOCELLULOLYTIC ACTIVITY OF *Ganoderma* spp. STRAINS ISOLATED FROM THE CENTRAL REGION OF VERACRUZ, MEXICO †

[ACTIVIDAD LIGNOCELULOLÍTICA DE CEPAS DE *Ganoderma* spp. AISLADAS DE LA REGIÓN CENTRAL DE VERACRUZ, MÉXICO]

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SUMMARY

Background. Fungi of the genus *Ganoderma* cause white rot of wood in forest trees and trees of economic importance due to the variety of lignocellulolytic enzymes they produce, which may be widely applied for environmental, food production, and biotechnological purposes, among others. **Methodology**. Fourteen strains of the genus *Ganoderma* isolated from the central region of Veracruz were studied. Qualitative assays were conducted in Petri dishes to determine the production of cellulase, laccase, and peroxidase enzymes in media with CMC, ABTS, and Azure B, respectively. Activity indexes were calculated according to the formation of discoloration and oxidation halos. Five strains with significant activity were selected, which were used for the quantitative determination of cellulase, laccase, lignin peroxidase, and manganese peroxidase activities using crude extracts obtained from liquid cultures in flasks with minimal medium by spectrophotometric assays with CMC, ABTS, Azure B, and manganese sulfate as reaction substrates. **Results.** The qualitative assays showed cellulase and laccase activity but not peroxidase activity in the studied strains. The quantitative assays showed cellulase, laccase, and lignin peroxidase activity but not manganese peroxidase activity, where laccase activity was the most relevant. The strains *G. weberianum* GV26 and *Ganoderma* sp. GV11 showed the highest laccase production on the 16th day of culture at 27±1 °C, with 102.8 and 106.2 U/L, respectively. **Implications.** The strains with significant enzymatic activities are candidates for further studies of optimization, purification, and bioremediation of recalcitrant xenobiotic compounds. **Conclusion.** The strains *Ganoderma* GV11 and GV26 are a potential source of lignocellulolytic enzymes that could be applied in biotechnological processes.
Key words: White rot; Cellulases; Laccases; ABTS; Azure B.

RESUMEN

Antecedentes. Los hongos del género *Ganoderma* son causantes de la pudrición blanca de la madera en árboles forestales y de importancia económica, debido a la variedad de enzimas lignocelulíticas que producen, las cuales pueden tener una amplia aplicación en el área ambiental, alimentaria, biotecnológica, entre otras. **Metodología**. Se estudiaron 14 cepas del género *Ganoderma* aisladas de la región central de Veracruz, mediante ensayos cualitativos en caja de Petri se determinó la producción de enzimas celulasa, lacasa y peroxidasa, en medios con CMC, ABTS y Azure B, respectivamente; con lo que se calcularon los índices de actividad de acuerdo a la formación de halos de decoloración y oxidación. Se seleccionaron cinco cepas con actividad significativa a las

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cuales se les realizó la determinación cuantitativa de las actividades celulasa, lacasa, lignino peroxidasa y manganeso peroxidasa, utilizando extractos crudos obtenidos de los cultivos líquidos en matraces con medio mínimo, esto mediante ensayos espectrofotométricos utilizando CMC, ABTS, azure B y sulfato de manganeso como sustratos de reacción. **Resultados.** En los ensayos cualitativos se observó actividad celulasa y lacasa pero no actividad peroxidasa en las cepas estudiadas. Por otro lado, en los ensayos cuantitativos se observó actividad celulasa, lacasa y lignina peroxidasa, pero no manganeso peroxidasa, siendo la actividad lacasa la más relevante; las cepas *G. weberianum* GV26 y *Ganoderma* sp. GV11 obtuvieron la mayor producción lacasa a los 16 días de cultivo, a 27±1 °C, con 102.8 y 106.2 U/L, respectivamente. **Implicaciones.** Las cepas con actividades enzimáticas significativas son candidatas para continuar con estudios de optimización, purificación y bioremediación de compuestos xenobióticos recalcitrantes. **Conclusión.** Las cepas de *Ganoderma* GV11 y GV26 son fuente potencial de enzimas lignocelulolíticas que podría tener una aplicación en procesos biotecnológicos.
Palabras claves: Pudrición blanca; Celulasas; Lacasas; ABTS; Azure B.

INTRODUCTION

Fungi of the genus *Ganoderma* P. Karst. 1881, which are considered basidiomycetes of the order Poryporales belonging to the family Ganodermataceae, are found in tropical and temperate regions worldwide (Moncalvo and Ryvarden, 1997; Cao *et al.,* 2012). This genus exhibits a wide spectrum of morphological variability, since some characteristics may change according to the substrate or geographic zone, which, along with the excessive use of synonyms and generalized names, have made its identification difficult and inconsistent, and it has thus been a taxonomically confusing genus since its establishment (Wang and Yao 2005; Cabarroi-Hernández *et al.*, 2019; Du *et al.*, 2019). This genus includes 181 accepted species worldwide (He *et al.,* 2022) and the mycological databases Index Fungorum (Index Fungorum, 2024) and MycoBank (Mycobank, 2024) show 495 and 539 records, respectively. There are several reports of the presence of some species of this genus in Mexico, such as the work by Mendoza *et al.*, (2011), who reported the presence of approximately 16 species of *Ganoderma*. Moreover, Torres-Torres *et al.,* (2015) mention 30 species of the subgenus *Ganoderma,* while Cappello-García *et al.,* (2023) found 29 species, where the states of Sonora and Veracruz were found to have a higher number of records and diversity of species, particularly the presence of *G. lobatum, G. weberianum, G. curtisii,* and *G. oerstedii* in the central region of Veracruz (Espinosa-García *et al.,* 2021).

Some species of *Ganoderma* have been used in traditional eastern medicine for two thousand years (Loyd *et al*., 2018; Cao *et al.,* 2018). These species are known for their production of secondary metabolites with anticancer, antihypertensive, anti-inflammatory, antioxidant, antibiotic, and antiviral activities, among others, which make them potential sources of medications and nutraceuticals (Suárez-Medellín *et al.,* 2012; Cao *et al.,* 2018). However, fungi of this genus are parasitic in nature and are responsible for white rot in live forest trees and trees of economic importance such as *Areca catechu* (betel nut palm), *Hevea brasiliensis* (rubber tree), and *Elaeis guineensis* (oil palm) (He *et al.,* 2022); the latter being mostly affected by *G. boninense*, which causes the death of up to 80% of plantations (Jazuli *et al.,* 2022; Khoo and Chong, 2023). The rot occurs due to the degradation of lignin, cellulose, and other wood components by lignocellulolytic enzymes (Falade *et al.,* 2017). The degradation of these components involves the participation of extracellular enzymes, such as laccase (EC 1.10.3.2), manganese peroxidase (EC 1.11.1.14), and lignin peroxidase (EC 1.11.1.13), among others, which belong to the group of lignin-modifying enzymes. These enzymes are characterized by their non-specificity (Kirk, 1987; Dávila and Vázquez-Duhalt, 2006), and thus, apart from degrading lignin, they can also act on other substrates, which allows their application in biotechnological processes that range from food processing and biopulping and biobleaching (Liew *et al.,* 2010; Wan and Li, 2012; Peralta *et al.,* 2017) to the transformation and degradation of various organic contaminants and xenobiotic compounds (Zhou *et al.,* 2012; Viswanath, 2014; Agrawal *et al.,* 2018; Singh *et al.,* 2019; Tuomela and Hatakka, 2019; Vishnoi and Dixit, 2019; Ipeaiyeda *et al.,* 2020).

The study of native species of *Ganoderma* broadens the knowledge of this genus in Mexico, particularly its biotechnological importance, such as its potential enzymatic production and the different areas where these enzymes can be used. Given that Veracruz is one of the states of Mexico with different reported species, it is relevant to conduct enzymatic bioprospecting studies. Considering the above, the present study aimed to assess the capacity to produce the main

extracellular lignocellulolytic enzymes of *Ganoderma* strains obtained from the central region of the state of Veracruz, Mexico.

MATERIALS AND METHODS

Microorganisms and culture conditions

Fourteen *Ganoderma* strains were studied with the following codes and GenBank accession numbers: *G. curtisii* GH16012 (MT254976), *Ganoderma* sp. GH16013, *G. curtisii* GH16015 (MT254977), G. *curtisii* GH16018 (MT254978), *G. curtisii* GH16019 (MT254979), *G. curtisii* GH16023 (MT254980), *Ganoderma* sp. GV11, *Ganoderma* sp. GV13, *G. tuberculosum* GV21 (MT232639), *Ganoderma* sp. GV22, *G. weberianum* GV26 (MT232635), *Ganoderma* sp. GV29, *G. weberianum* GV31 (MT232642) and *Ganoderma* sp. GV81(**Figure 1**), which belong to the collection of the Applied Mycology Research Center, were studied and isolated from different substrates in the central region of the state of Veracruz (Serrano-Márquez *et al.*, 2021, Espinosa-García *et al*., 2021). The strains were cultured in potato dextrose agar (PDA) at 27 °C ± 1 in darkness for seven days for their reseeding and maintenance.

Qualitative assay of cellulase activity

The fungi were cultured in a culture medium with agar-agar (1.7%, w/v) and carboxymethylcellulose (CMC 1%, w/v) as the only source of sterilized carbon at 121 °C for 15 minutes. The 14 strains were inoculated in Petri dishes in triplicate and incubated for 5 days at 27 °C ± 1. The azo dye Congo red (0.5 %, w/v) was used to reveal the cellulolytic activity by pouring it in the Petri dishes for 15 minutes, which were then washed with a 1 N NaCl solution (Meddeb-Mouelhi *et al.,* 2014; Coniglio *et al.,* 2016). Subsequently, mycelial growth and the formation of cellulase discoloration halos were measured. The obtained values were used to calculate the potency index (PI) by dividing the diameter of the discoloration halo by the mycelial growth diameter of each strain (Chan-Cupul *et al.,* 2016).

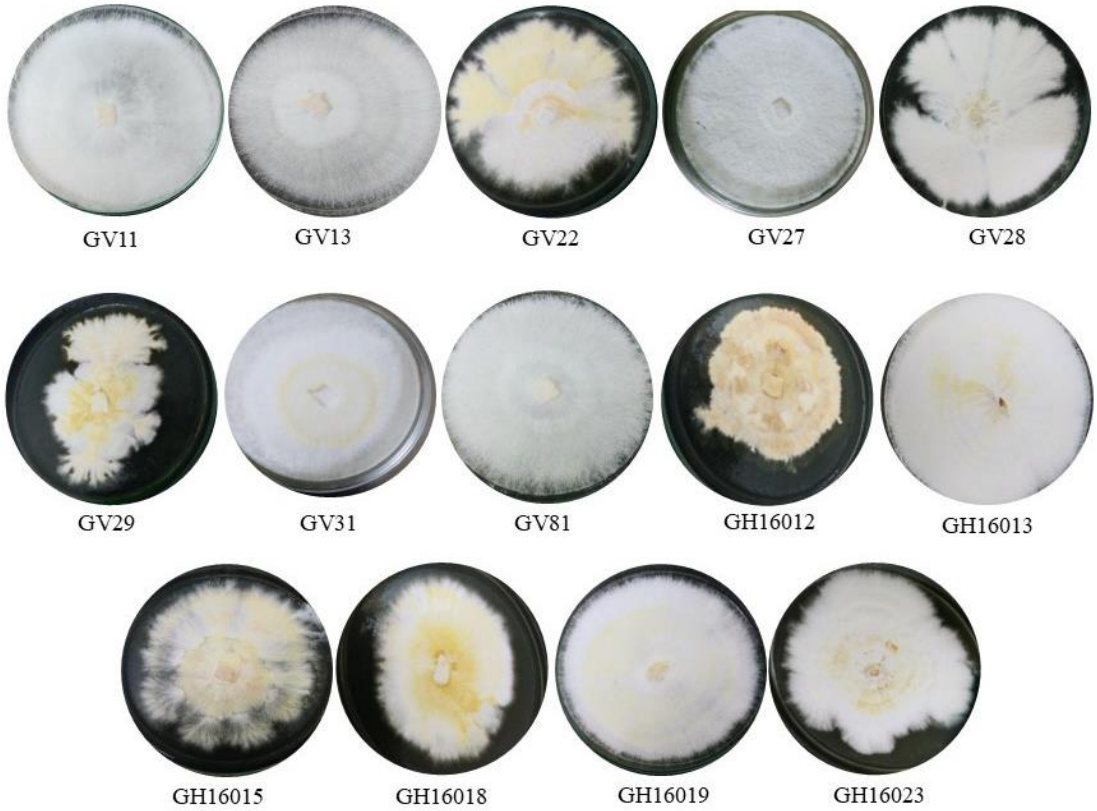

Figure 1. Mycelial growth of the 14 *Ganoderma* spp. strains used in the present study.

Qualitative assay of ligninolytic activities

For peroxidase activity, the heterocyclic dye Azure B (5 mM) was used in a PDA medium (Monotya *et al.*, 2014). In the case of laccase activity, 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) was used in a medium with the following composition (g/L): glucose, 15; KH_2PO_4, 1.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2$, 0.1; $(NH_4)_2SO_4$, 0.05; $ZnSO_4$, 0.03; $MgSO_4$, 0.002; $MnSO_4$, 0.002; $CuSO_4$, 0.002; $FeSO_4$, 0.01; and agar, 20 (Jo *et al.*, 2009, Chan-Cupul *et al.*, 2016).

The culture media were sterilized, and the 14 strains were inoculated in each medium in triplicate and incubated for 15 days at 27 °C ± 1. Subsequently, mycelial growth and the formation of peroxidase discoloration halos and laccase oxidation halos were measured, and the PI was calculated by dividing the diameter of the formed halo by the mycelial growth diameter of each strain for both determinations (Chan-Cupul *et al.*, 2016).

Quantitative assay of enzymatic production

The *Ganoderma* strains were cultured in liquid medium with the following composition (g/L): glucose, 15; KH_2PO_4, 1.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2$, 0.1; $(NH_4)_2SO_4$, 0.05; $ZnSO_4$, 0.03; $MgSO_4$, 0.002; $MnSO_4$, 0.002; $CuSO_4$, 0.002; $FeSO_4$, 0.01; and agar, 20 (Jo *et al.*, 2009; Chan-Cupul *et al.*, 2016) for cellulase, laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) enzyme production. Erlenmeyer flasks (500 mL) were inoculated in triplicate with 120 mL of culture medium for each studied strain with mycelium discs (5 mm in diameter) and incubated for 20 days at 27 ± 1 °C in shaking conditions at 140 rpm and in darkness.

Cellulase activity of crude enzyme extract

One milliliter of liquid medium was centrifuged at 8 000 rpm for 10 minutes. The supernatant was used to determine the cellulase activity using 50 µL of crude enzyme extract and CMC (1%, w/v) as a substrate in 100 µL of sodium citrate buffer (50 mM) at a pH of 4.8. After 60 minutes of incubation at 50 °C, 300 µL of dinitrosalicylic acid (DNS) were added to each reaction, which was then incubated at 95 °C for 5 minutes. Finally, a 36 µL aliquot of each sample was transferred to the wells of a flat-bottom plate containing 160 µL of H_2O, and absorbance (540 nm) was measured in a Labsystems Multiskan MCC/340 microplate reader (Yu *et al.*, 2016). One unit of enzymatic activity (U) was defined as the amount of enzyme produced by 1 µmol of reducing sugars in one minute.

Ligninolytic activities of crude enzyme extract

For laccase activity, 16 µL of crude enzyme extract were used with 16 µL of ABTS (3 mM) as a substrate in 150 µL of sodium acetate buffer (100 mM) at a pH of 4.5. The change in absorbance (405 nm) was monitored in a microplate reader (Labsystems Multiskan MCC/340) during the first 5 minutes of the reaction (Heinzkill *et al.*, 1998; Chmelová and Ondrejovic, 2014). One laccase unit (U) was defined as the amount of enzyme required to oxidize 1 µmol of ABTS in one minute.

Lignin peroxidase activity was determined with 0.5 mL of crude enzyme extract in 1.5 mL of sodium tartrate buffer (50 mM) with 0.5 mL of Azure B (32 µM). The reaction was started by adding 0.5 mL of H_2O_2 (2 mM) (Archibald 1992; Arora and Gill, 2001). The absorbance reading (650 nm) was monitored in a UV/Vis spectrophotometer (PerkinElmer Lambda 265 UV/Vis). One unit of enzymatic activity (U) is equivalent to a decrease of 0.1 absorbance units per minute and per mL of crude extract.

Finally, MnP activity was determined with 50 µL of crude enzyme extract in 450 µL of sodium malonate buffer (50 mM) and manganese sulfate (1 mM). The reaction was started by adding H_2O_2 (0.1 mM). The absorbance reading (270 nm) was monitored in a PerkinElmer Lambda 265 UV/Vis spectrophotometer (Wariishi and Gold, 1990; Wariishi *et al.*, 1992; Huy *et al.*, 2017). One unit of enzymatic activity (U) was defined as the amount of enzyme required to produce 1 µmol of oxidized substrate per minute under the experimental conditions.

Statistical analyses

Analyses of variance and multiple comparison tests were performed after testing the assumptions of normality and homoscedasticity. A 95% confidence level was used to determine the maximum production values of enzymatic activity that were significantly different between the different studied strains. All statistical analyses were performed in GraphPad Prism 8.

RESULTS AND DISCUSSION

Enzymatic activities in Petri dishes

Of the 14 strains studied, five showed enzymatic activity in the qualitative assay, which was corroborated by the presence of cellulase discoloration halos and laccase coloration halos (**Figure 2**). No formation of peroxidase discoloration halos was observed and thus this enzymatic activity was assumed to be absent. **Table 1** shows the PI of the strains with cellulase and laccase activity based on the differences of the coloration/discoloration halos.

Table 1. Cellulase and laccase enzymatic activity of *Ganoderma* strains with halo formations in Petri dishes.

Ganoderma strains	Potency Index (PI)	
	Cellulase activity	Laccase activity
GH16015	1.41 (± 0.206) [a]	1.71 (± 0.085) [a]
GH16023	5.01 (± 0.101) [b]	5.12 (± 0.335) [b]
GV11	1.74 (± 0.269) [a]	1.76 (± 0.129) [a]
GV26	2.06 (± 0.400) [a]	1.76 (± 0.073) [a]
GV81	1.59 (± 0.433) [a]	1.52 (± 0.113) [a]

Values with different superscript letters are significantly different ($p < 0.05$).

In the case of cellulase activity, the Kruskal-Wallis test showed significant differences with a $p < 0.009$, and the multiple comparisons analysis indicated that the strain GH16023 differed significantly from the other strains with a PI of 5.01, which was the highest value among the five selected strains. Laccase activity was confirmed by the presence of ABTS oxidation halos, and the Kruskal-Wallis test showed significant differences between strains with a $p < 0.009$, where the multiple comparisons indicated that GH16023 was the strain with the highest laccase activity compared to the other strains, with a PI of 5.12, which was the highest value among the evaluated strains, as with cellulase activity.

The PI values allowed us to select the strains with the highest capacity for cellulase and laccase enzyme production through the formation of halos in Petri dishes by using substrates that allow the observation of such activities, which is a frequently used technique in processes of selection of organisms with enzymatic capacities of interest (Chan-Cupul *et al*., 2016; Coronado-Ruiz *et al.*, 2018; Kaur *et al.*, 2018; Sánchez-Corzo *et al.*, 2021). The result obtained from the observations of cellulase activity, based on the PI in the medium with CMC, showed that only one of the 14 strains studied showed a greater potential as a producer of extracellular cellulase. The difference observed in cellulase activity between strains may be mainly due to interspecific variation, since the strains belonged to different species. However, it should be taken into account that these variations in enzymatic production may also be related to other factors, as variation in cellulolytic activity has also been observed between isolates of different fungi of the same species (Pedersen *et al.*, 2009).

In the case of ligninolytic activities, like with cellulase activity, not all strains had the capacity to produce laccase and peroxidases. Regarding peroxidase activity, authors such as Montoya *et al.*, (2014), Pingili *et al.*, (2017), and Thiribhuvanamala *et al.*, (2017) have shown that the dye Azure B is widely used for the qualitative determination of enzymes such as LiP and MnP by observing its discoloration. However, in the present study, we did not observe the formation of discoloration halos or the discoloration of the culture medium. Thus, the absence of Azure B discoloration halos may be directly related to the

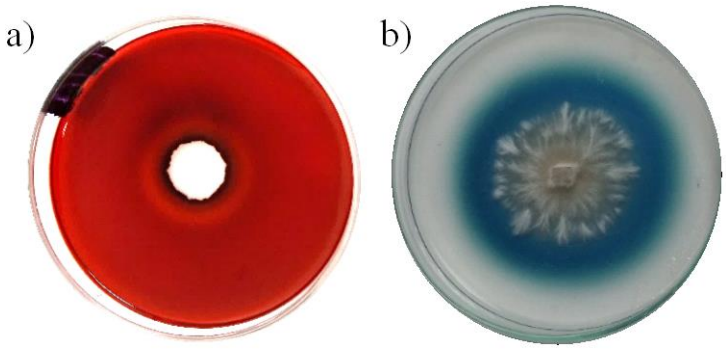

Figure 2. Enzymatic activities in Petri dishes. a) Strain with cellulase activity, b) strain with laccase activity.

absence of the peroxidase enzymes of interest during the growth of the strains in this medium. Laccase activity was detected by the formation of green-blueish oxidation halos around the mycelial colony, which is characteristic of white-rot fungi such as *Ganoderma* sp. (Montoya *et al.,* 2014; Chan-Cupul *et al.,* 2016). However, only five strains had PI values, which indicates the difference in enzymatic capacity between the 14 strains studied.

The variation in the presence and absence of enzymatic activity in the semiquantitative assays of the studied strains may be related to the different characteristics between *Ganoderma* species. Their growth and enzymatic production are related to their host species in nature, the environmental conditions, the nutrient concentration, and, in the case of ligninolytic activity, the presence of metal ions (Stoilova *et al.,* 2010; Chan-Cupul *et al.,* 2016; Saptarini and Hadisoebroto 2017).

Lignocellulolytic activity in liquid fermentation

The culture of the selected strains was used to prepare a liquid fermentation in a basal medium. Cellulase, laccase, lignin peroxidase, and manganese peroxidase activities were determined with crude extracts after 20 days of culture. **Figure 3** shows the obtained activities over time. A two-way analysis of variance (ANOVA) was performed to assess the effect of day, strain, and the interaction day-strain on enzymatic activity and determine the presence of significant differences. The results of the two-way ANOVA showed that strain, fermentation day, and their interaction had a significant effect on the three enzymatic activities (cellulase, laccase, and LiP), indicating significant differences (all with a $p < 0.0001$).

The behavior of cellulase activity was similar in the five strains, but GH16015 and GH16023 showed a higher cellulase production with 0.165 U/mL and 0.183 U/mL, respectively (**Figure 3a**). Laccase, LiP, and MnP activities were determined for the quantification of ligninolytic enzymes. In the case of laccase activity, GV26 and GV11 showed the highest activity on the 16th day with 102.8 and 106.2 U/L, respectively (**Figure 3b**). No MnP activity was observed in any of the five strains during the established time. In the case of LiP, GH16015 showed the highest activity of the five strains on the 16th day with 2.56 U/L, followed by GV26 with 1.89 U/L on the 17th day (**Figure 3c**).

Based on the behavior and values of the quantified enzymatic activities, the strain GV26 was selected for its enzymatic potential. Espinosa-García *et al*. (2021) have previously reported the antiproliferative activity of this strain and it has been taxonomically and molecularly identified as *G. weberianum*.

The variations in enzymatic production observed between strains in the present study may be explained by changes produced by extrinsic factors during the fermentation time (culture medium, temperature, pH, etc.), as well as intrinsic factors linked to the complex fungal metabolism (Salmon *et al.,* 2014). These variations in the physiology of the strains occur even within the same species. The maximum laccase activity values were observed on the same day (day 16). Enzyme production can vary according to the studied species and its fungal metabolism, as well as to the composition of the culture medium and the presence of different substrates and/or inducers in the medium, among others (Dinis *et al.,* 2009; de Menezes *et al.,* 2016; Zhou *et al.,* 2018; Júnior *et al.,* 2022). Thus, the enzyme production times of *Ganoderma* strains can vary between five and 20 days of culture (Elissetche *et al.,* 2006; Dinis *et al.,* 2009).

The five strains that showed a PI in the Petri dish assays were selected for the liquid fermentation process, where differences were observed in the behavior of the enzymatic activity when performing the qualitative and quantitative analyses. The strain with the highest cellulase activity in the Petri dish and liquid fermentation analyses was GH16023. The strain GH16023 also showed the highest PI for laccase activity in the Petri dish, while GV11 and GV26 showed the highest values in the liquid fermentation. It is important to mention that it was not possible to quantify peroxidase activity (which includes LiP and MnP) in the Petri dishes, and LiP, but no MnP, activity was observed in the liquid fermentation. This may be related to the culture medium, since PDA and Azure B were used in the Petri dishes for peroxidase activity, while the liquid fermentations were prepared in a basal culture medium with different trace elements, which have been reported to affect the growth and enzymatic production of fungal strains (Chauhan, 2019).

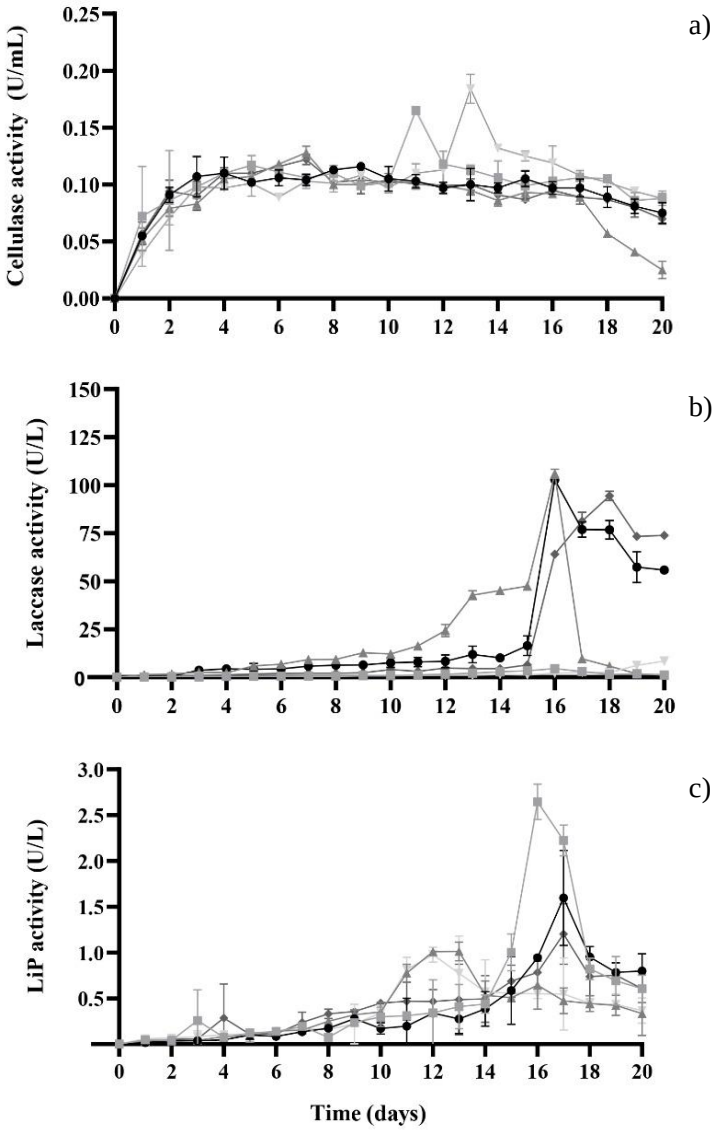

Figure 3. Enzymatic activities of the crude extracts of the five *Ganoderma* strains obtained in a basal medium during the fermentation period. a) Cellulase, b) Laccase, and c) LiP. (■) GH16015, (▼) GH16023, (▲) GV11, (●) GV26, and (♦) GV81.

There are reports of the absence of one or more of the main ligninolytic activities in white-rot fungi studies. For example, Hernández-Luna *et al*. (2017) conducted a decolorizing study with basidiomycetes collected in the north of Mexico and found ligninolytic (laccase, MnP, and LiP) activity in *Trametes villosa* (CS5) and *T. maxima* (CU1) strains in qualitative assays in Petri dishes, but quantitative assays showed laccase activity in both strains, MnP activity only in the CS5 strain, and LiP activity was not observed in any of the strains.

Some studies have shown variations in ligninolytic enzyme production in *Ganoderma* depending on the substrate, culture conditions, and even the environment where the fungi are found. Mendoza-Arceo *et al*. (2024) reported the absence of some enzymatic activities (laccase, MnP, and LiP) that they quantified in *Ganoderma* spp. strains from two different environments: urban and ruderal. In the case of the ruderal environment, three of the seven strains studied showed laccase, MnP, and LiP activities, while the other four strains did not show MnP activity. Silva *et al.* (2005) studied four *Ganoderma* spp. strains (CB364, GASI3.4, CCB209, and GASI2), where only two produced

laccase, MnP, and LiP, while the other strains only produced laccase. The strains GASI3.4 and CCB209 showed the three enzymatic activities and were cultured in three different liquid media: one without supplementation, one supplemented with wheat bran, and one supplemented with propanil. The strain GASI3.4 showed the three enzymatic activities at different time intervals, showed no MnP activity in the second medium, and no LiP or MnP activity in the third medium. The strain CCB209 exhibited the three enzymatic activities in the medium without supplementation and the medium supplemented with propanil, but MnP activity was absent in the medium with wheat bran. This indicates that, even though *Ganoderma* and other white-rot fungi have the capacity to produce ligninolytic enzymes, they may not produce one of these enzymes if it is not essential for the degradation of the substrate where they are found.

Cellulase production was similar among the five strains studied during the liquid fermentation process, with the strain GH16023 showing the highest production (0.183 U/mL) at 13 days of incubation. This agrees with that reported by Dinis *et al*. (2009), who quantified the production of cellulases in white-rot basidiomycete fungi in CMC, where the maximum activity value (0.130 U/mL) of *G. applanatum* was observed on day 14. Saptarini and Hadisoebroto (2017) cultured *G. applanatum* and *G. tropicum* at different pH values and temperatures using CMC for cellulase activity, where *G. applanatum* exhibited a maximum enzymatic activity value of 0.109 U/mL at a pH of 10 and of 0.184 U/mL at 45 °C, while the maximum activity value for *G. tropicum* was of 0.131 U/mL at a pH of 10 and of 0.110 U/mL at 45 °C. Hu *et al.* (2019) studied the regulation of the Glsnf1 gene for cellulase degradation in *G. lucidum* using CMC as a substrate in the enzymatic determinations and reported cellulase activity values from 0.050 to 0.200 U/mL in treatments with different concentrations of the activator 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) of the studied gene.

In the case of the ligninolytic enzymes during the liquid fermentation process, we observed the presence of laccase and LiP, where the former was the main oxidative enzyme produced by three of the five strains studied, while LiP was produced at a lower concentration and in a similar way by the five strains. The days when the highest laccase production values of the five strains were observed differ from those reported by different authors (Montoya *et al.*, 2014, Shrestha *et al.*, 2014, Chan-Cupul *et al.*, 2016 and Qin *et al.*, 2019), who mention that the production of laccase in white-rot lignicolous fungal species, starts to increase on the sixth day of the fermentation process, whereas we observed an increase in the activity of this enzyme on the 13th day and the highest production was observed from the 16th to the 18th day, which agrees with that reported by Dinis *et al.*, 2009 and de Menezes *et al.* (2016). The observed difference in enzymatic production may be related to the culture conditions, where the medium composition and substrates used are important elements (Silva *et al.*, 2005; Elissetche *et al.*, 2006). With respect to the medium composition, different carbon sources, the amount of nitrogen, and the addition of amino acids, vitamins, and trace elements such as Fe, Zn, $CaCl_2$, $MgSO_4$, and KH_2PO_4 can limit or induce the production of ligninolytic enzymes (Erden *et al.*, 2009; Levin *et al.*, 2010; Stajić *et al.*, 2013, Cha-Cupul *et al.*, 2016; Chauhan, 2019; Shehnaz and Prasher, 2022).

In the case of ligninolytic enzyme production with crude extracts, the behavior of laccase activity was similar in the strains GV11, GV26, and GV81, with maximum activity values of 94.3, 102.8, and 106.2 U/L, respectively. De Menezes *et al.* (2016) reported a maximum laccase activity value of 13.80 U/L in *G. lucidum* cultures with a palm tree native to Brazil called licuri (*Syagrus coronata*) as a substrate. Elissetche *et al.* (2006) obtained 2 U/L of laccase activity in *G. australe* using a synthetic culture medium and Rodrigues *et al.* (2019) obtained a laccase activity value of 44 U/L in GYP (glucose-yeast-peptone) medium with ferulic acid as inducer in a *G. lucidum* culture where different inducers were studied, and ferulic acid was found to be the best inducer at 2 mM. Compared with the studies mentioned above, this study presented higher enzymatic values with *Ganoderma* strains grown in a medium with a different substrate and without supplements or inducers.

The behavior of LiP activity was similar in the five strains studied during the liquid fermentation process, where GH16015 showed the highest production with 2.56 U/L at 16 days of incubation, which agrees with other *Ganoderma* studies. Silva *et al*. (2005) mentions the presence of LiP activity in the GASI3.4 and CCB209 strains of this genus with values of 18.851 and 2.126 U/L, respectively. Mendoza-Acero *et al.* (2024) report the presence of LiP in strains collected from an urban and a ruderal environment, where the lowest LiP activity value in the strains from the ruderal environment was 6.93 U/L and the highest was 9.95 U/L, and the lowest value in the strains from the urban

environment was 0.36 U/L and the highest was 2.29 U/L.

In addition to enzymatic studies, it is important to study the metabolism of *Ganoderma*, which is diverse due to its metabolic complexity. Trigos and Suárez (2011) reviewed the main biologically active metabolites of the genus and note their polarity and the importance of ganoderic acids, polysaccharides, peptides, and proteins, which may have therapeutic applications. There are also studies that have examined the production of secondary metabolites with antioxidant, antiproliferative, and antibacterial activity in strains isolated from the central region of Veracruz, Mexico (Espinosa-García *et al*, 2021; Serrano-Márquez *et al*., 2021).

CONCLUSIONS

Fungi of the genus *Ganoderma* are best known for their medicinal importance, particularly *G. lucidum*. However, there are species that have been poorly studied regarding the bioprospection of their extracellular lignocellulolytic activity. There are several species endemic to Veracruz that could have a significant enzymatic potential. The strains isolated from the central region of this state used in the present study showed enzymatic activity, particularly laccase activity, which is currently used in processes of bioremediation of recalcitrant xenobiotic compounds, paper bleaching and biopulping, food production, biofuel production, among others. The results obtained with the strain *G. weberianum* GV26 could be complemented with further studies on the optimization of enzymatic production that evaluate different substrates, temperatures, pH values, inducers, C/N ratios, among others, and even studies on enzymatic purification. All of this with the purpose of providing a biotechnological application.

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