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Phytase production by white-rot fungi cultivated in liquid medium with particulate agro-industrial byproducts

Produção de fitase por fungos da podridão branca cultivados em meio líquido com subprodutos agroindustriais particulados

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ABSTRACT

This study aimed to screen phytase-producing basidiomycetes by submerged cultivation (SmC) with particulate agro-industrial byproducts and evaluate the enzyme's thermal and pH stability. Five among eight fungal species produced high phytase activity (> 300 U mL^{-1}) after seven days in SmC. *Ganoderma* sp., the best phytase producer (1130 U mL^{-1}), was grown in a liquid medium with particulate agro-industrial byproducts (coffee husks, citric pulp, sugarcane bagasse, and soybean hulls). Soybean hulls increased phytase activity (16%) and productivity (2.3-fold). The residual phytase activity was determined after 120 min incubation in different pH (pH 3, 5, 7, and 9) and temperatures (30, 50, and 70 ºC). Phytase activity

remained stable at pH ranging from 3 to 9, with 100% activity retention after 120 min at pH 7. Furthermore, Phytase retained more than 60% activity when incubated for 120 min from 30 ºC to 70 ºC. Our findings support future studies using *Ganoderma* sp. and particulate soybean hulls for phytase production for potential use in the animal feed industry.

Keywords: Basidiomycete; *Ganoderma*; Phosphohydrolase; Submerged cultivation.

RESUMO

Este trabalho teve como objetivo selecionar basidiomicetos produtores de fitase por cultivo submerso (SmC) com subprodutos agroindustriais particulados e avaliar a estabilidade térmica e de pH da enzima. Cinco entre oito espécies de fungos produziram alta atividade de fitase (> 300 U mL^{-1}) após sete dias em SmC. *Ganoderma* sp., o melhor produtor de fitase (1130 U mL^{-1}), foi cultivado em meio líquido com subprodutos agroindustriais particulados (cascas de café, polpa cítrica, bagaço de cana-de-açúcar e casca de soja). A casca de soja aumentou a atividade da fitase (16%) e a produtividade (2,3 vezes). A atividade residual da fitase foi determinada após 120 min de incubação em diferentes pH (pH 3, 5, 7 e 9) e temperaturas (30, 50 e 70 ºC). A fitase permaneceu estável em pH variando de 3 a 9, com 100% de retenção de atividade após 120 min em pH 7. Além disso, manteve mais de 60% de atividade quando incubada por 120 min de 30 ºC a 70 ºC. Nossos resultados suportam estudos futuros usando *Ganoderma* sp. e casca de soja para produção de fitase para uso potencial na indústria de ração animal.

Palavras-chave: Basidiomiceto; *Ganoderma*; Fosfohidrolase; Cultivo submerso.

INTRODUCTION

Phytic acid has been reported with anti-nutritional activity because it chelates cations, an issue for swine and poultry farming. Moreover, the phytate in the livestock feed alters the secretion of endogenous compounds such as animal digestive enzymes, HCl, and mucin, which further reduces nutrient availability (VASUDEVAN et al., 2019; WIDDERICH et al., 2022). The feed costs for swine and poultry production account for about 60–70% of total livestock production costs, and around 25% of the feed is released through feces, a difficult-to-treat effluent with a high environmental impact (RAVINDRAN, 2013). Thus, it is essential to use phytases (phosphohydrolases) (ZHU et al., 2011) a necessary enzyme in the livestock feed, to increase the nutrient availability in animal feed. Phytases have been used, mainly in poultry diets, for over 20 years and account for 83.6% sales of the market of animal feeding enzymes, estimated to be USD 2.3 billion by 2026 (AMERAH et al., 2011).

Phytase production is affected by variables such as the microbial species and strain, cultivation medium composition, inducers or inhibitors, pH, temperature, salinity, and the cultivation system (JATUWONG et al., 2020). Although there are different phytase sources, the most commercially used ones are from fungi because they produce stable, extracellular phytases under broad temperature and pH ranges (SINGH; SATYANARAYANA, 2015). White-rot basidiomycetes can be a promising source of phytases. However, they represent approximately 3,000 species, and only 60 of them are commercially cultivated (CHANG; MILLES, 2004) for a world market of around 35 billion kg of mushrooms per year (ROYSE et al., 2017). Despite the great diversity, few basidiomycetes have been studied for phytase production by submerged cultivation, such as *Ganoderma* sp. (SALMON et al., 2016) and *Grifola frondosa* (HUANG et al., 2018).

Submerged cultivation has advantages to enzyme production. These include greater homogeneity and easy control of cultivation variables, simplified recovery of biomass and enzymes (ELISASHVILI et al., 2008), reuse of cultivation medium with a lower environmental impact, and various equipment to scale up the process (CERDA et al., 2019). Furthermore, the submerged cultivation with the addition of particulate solids from agro-industrial byproducts has shown promising results for enzyme production (ALMEIDA et al., 2018; CARDOSO et al., 2018). Brazil has one of the biggest world food industries, generating several wastes that could become an environmental challenge (RAVINDRAN et al., 2018). These wastes have significant carbon and nitrogen contents and could produce biomass or bio compounds of economic interest, such as phytases. This study aimed to screen phytase-producing white-rot basidiomycetes by submerged cultivation (SmC) with particulate agro-industrial byproducts and evaluate phytase thermal and pH stability. A previous screening in solid medium containing phytate was utilized to initial select strains for phytase production. As far as we know, this is the first report of phytase

production in SmC for *Lentinus berteroi*, *Lentinus crinitus*, and *Pycnoporus sanguineus* (Polyporales) and very high phytase activity reported for *Ganoderma* sp. (Polyporales) that represents an essential source of phytases.

MATERIAL AND METHODS

Microorganisms

Eight white-rot basidiomycete strains from Agaricales Order *Agaricus subrufescens* Peck U2-4 (*Agaricus blazei* Murrill; *Agaricus brasiliensis* Wasser et al.), *Lentinula edodes* (Berk.) Pegler U6-11, *Pleurotus eryngii* (DC.) Quél. U12-3, *Pleurotus ostreatus* (Jacq.) P. Kumm. U6-9, and from Polyporales Order *Ganoderma* sp. U13-3, *Lentinus berteroi* (Fr.) Fr. U13-5, *Lentinus crinitus* (L.) Fr. U9-1, and *Pycnoporus sanguineus* (L.) Murrill U13-4 from the culture collection of the Molecular Biology Laboratory of the Paranaense University were evaluated. Mycelia were cultivated in the dark on 20 g L^{-1} malt extract agar (MEA) at 28 ± 1 ºC, and MEA discs (6 mm) with mycelia were used as inoculum for solid medium and liquid medium.

Screening of phytase-producing strains

The mycelial growth in a solid cultivation medium containing phytate was the criterion for screening strains with phytase activity. The fungi were grown in Petri dishes (9-cm diameter) containing agar-phytate medium composed of 5.0 g L^{-1} phytate, 2.0 g L^{-1} yeast extract, 2.0 g L^{-1} $NaNO_3$, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} KCl, 0.1 g L^{-1} $ZnSO_4.7H_2O$, 0.05 g L^{-1} $FeSO_4$, and agar 19.0 g L^{-1} (COBAN; DEMIRCI, 2014). One MEA disc with mycelia was inoculated in the center of the cultivation medium and kept at 28 ± 1 ºC in the dark for 21 days. Three measures of the colony diameter were taken with a caliper, and the strains with a growth rate greater than or equal to 3 mm day^{-1} were selected for the subsequent assays.

Phytase production in liquid medium

The strains were grown in a liquid medium to evaluate phytase production under submerged cultivation (SmC). The cultivation was carried out in conical flasks (125 mL) with 50 mL autoclaved (121 ºC for 20 min) cultivation medium containing 2.0 g L^{-1} yeast extract, 2.0 g L^{-1} $NaNO_3$, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} KCl, 0.1 g L^{-1} $ZnSO_4.7H_2O$, and 0.05 g L^{-1} $FeSO_4$ (SPIER et al., 2008). A phytate solution was filtered (0.22 μm pore membrane) and added to the previously autoclaved liquid medium to obtain 5.0 g L^{-1}. After inoculation with three MEA discs, the flasks were kept without agitation at 28 ± 1 ºC in the dark, and phytase activity was determined every seven days throughout 21 cultivation days.

The strain with the highest phytase activity in SmC with phytate was grown in the liquid medium added of particulate agro-industrial byproducts such as coffee husks (CH), citric pulp

(CP), sugarcane bagasse (SB), and soybean hulls (SH). The liquid medium was prepared as previously described but with the addition of 2.5 g L^{-1} of the cited agro-industrial byproducts. Liquid cultivation medium without agro-industrial byproducts was used as control.

Agro-industrial byproducts characterization

The CH, CP, SB, and SH were analyzed according to AOAC (2000). The total nitrogen content was determined by Kjeldahl's method AOAC 955.04D (2000); the moisture was estimated according to AOAC 969.35 (2000), and the ashes were determined by gravimetric method AOAC 969.36 (2000). Considering that 50% of the dry organic mass corresponds to the byproduct carbon mass, the results of the chemical analysis were used to calculate carbon-to-nitrogen (C/N) ratio (GERRITS, 1988). The chemical characteristics of the agro-industrial byproducts used in the liquid media are described in Table 1.

Table 1 - Chemical characterization of agro-industrial byproducts added to the liquid cultivation media for phytase production.

Agro-industrial byproduct	Ashes (%)	Carbon (%)	Nitrogen (%)	C/N ratio
Citric pulp (CP)	2.93 ± 0.15	42.15± 0.05	1.40 ± 0.03	30.11
Coffee husks (CH)	6.21 ± 0.14	40.45 ± 0.08	3.20 ± 0.14	12.63
Soybean hulls (SH)	12.13 ± 1.60	38.15 ± 1.93	1.34 ± 0.02	28.47
Sugarcane bagasse (SB)	2.62 ± 0.21	44.43 ± 0.48	0.89 ± 0.04	49.80

Phytase stability assays

The phytase pH stability was determined by measuring the residual enzymatic activity after incubating the enzyme at different pH. Aliquots of the liquid medium containing phytase were mixed (2:1, volume: volume) with pH buffer solutions such as sodium acetate buffer (pH 3 and pH 5), sodium phosphate buffer (pH 7), and glycine-NaOH buffer (pH 9). The mixtures were kept at room temperature for 120 min, and phytase activity was determined every 30 min. The thermal stability of phytase activity was determined by measuring the residual enzymatic activity after incubation of the enzyme at different temperatures. The liquid medium containing phytase was kept at 30 ºC, 50 ºC, and 70 ºC for 120 min, and phytase activity was determined every 30 min after the liquid medium had cooled at room temperature. Residual activity at the end of the 120-min incubation period was used to calculate half-life ($t_{1/2}$), according to the equation:

$$N = N_0\ (0.5)^{t/h}$$

Where N is the residual phytase activity, N_0 is the initial phytase activity, *t* is time (min) and *h* is the half-life or time (min) required for the phytase activity to be reduced by half.

Phytase activity assay

Phytase activity assay was carried out according to Harland and Harland (1980). Phytase activity was determined by the amount of orthophosphate generated in a reaction containing 1 mL sodium phytate (1.5 mM), prepared in sodium citrate buffer (pH 3), and 1 mL liquid medium kept at 50 °C for 10 min. The reaction was stopped by adding 1 mL trichloroacetic acid (10 g 100 mL^{-1}) to the mixture, 1 mL ultrapure water, and 5 mL Taussky-Shorr's reagent (TAUSSKY; SHORR, 1953). Orthophosphate absorbance was determined at 660 nm using a potassium phosphate calibration curve (R^2 = 0.989). Under experimental conditions, the phytase activity unit (U) was defined as the amount of enzyme required to release 1 μmol of inorganic phosphate per minute.

Statistical analysis

All the assays followed a completely random design with three replications. The results were evaluated using analysis of variance (ANOVA), and significant differences among arithmetic means were determined by the Scott-Knott test at 5% probability. Pearson correlation coefficient (r) between the C/N ratio of the agro-industrial byproduct and phytase activity was assessed.

RESULTS

All strains grew in the solid medium added with phytate; however, Polyporales strains had the highest mycelial growth rates, such as *P. sanguineus*, followed by *Ganoderma* sp., *L. crinitus*, and *L. berteroi* (Table 2). Agaricales strains had lower ($p \leq 0.05$) mycelial growth rate (from 1.1 ± 0.16 to 2.1 ± 0.08 mm day^{-1}) compared to Polyporales strains (from 3.3 ± 0.30 to 4.3 ± 0.13 mm day^{-1}) (Table 2). Thus, as Polyporales strains presented the greatest mycelial growth rate values in the cultivation medium with phytate compared to Agaricales strains, they were selected for the phytase production assays by SmC cultivation.

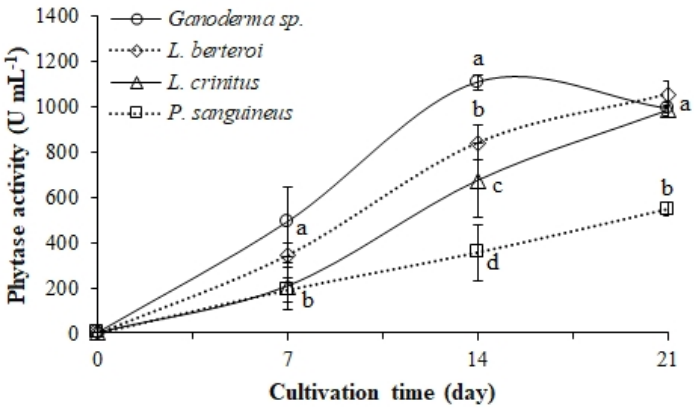
Ganoderma sp. showed the highest ($p \leq 0.05$) phytase activity throughout the cultivation period, with activity peak at 14 cultivation days (1108 ± 35 U mL^{-1}) (Figure 1). The second best phytase producer was *L. berteroi*, whose activity peak (1055 ± 56 U mL^{-1}) occurred after 21 cultivation days when *L. crinitus* also reached maximum phytase activity (984 ± 23 U mL^{-1}). The maximum phytase activity of *P. sanguineus* did not exceed 550 U mL^{-1}. Thus, *Ganoderma* sp. was selected for the next cultivation phase in a liquid medium with agro-industrial byproducts.

Table 2 - Mycelial biomass growth diameter (mean ± standard deviation) and mycelial growth rate of basidiomycete strains on agar-phytate medium (5.0 g L^{-1} phytate, 2.0 g L^{-1} yeast extract, 2.0 g L^{-1} $NaNO_3$, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} KCl, 0.1 g L^{-1} $ZnSO_4.7H_2O$, 0.05 g L^{-1} $FeSO_4$, and 19.0 g L^{-1}) at 28 ± 1 °C for 21 days.

Order	Strain	Code	Mycelial growth diameter (cm)	Mycelial growth rate (mm day^{-1})
Agaricales	*Agaricus subrufescens*	U2-4	4.33 ± 0.17[d]	2.1 ± 0.08[d]
	Lentinula edodes	U6-11	2.66 ± 0.29[e]	1.3 ± 0.13[e]
	Pleurotus eryngii	U12-3	2.38 ± 0.35[e]	1.1 ± 0.16[e]
	Pleurotus ostreatus	U6-9	4.24 ± 0.23[d]	2.0 ± 0.11[d]
Polyporales	*Ganoderma* sp.	U13-3	8.11 ± 0.12[b]	3.9 ± 0.05[b]
	Lentinus berteroi	U13-5	7.00 ± 0.69[c]	3.3 ± 0.30[c]
	Lentinus crinitus	U9-1	7.96 ± 0.26[b]	3.8 ± 0.12[b]
	Pycnoporus sanguineus	U13-4	8.95 ± 0.27[a]	4.3 ± 0.13[a]

*Values indicated by different letters in the column differ statistically by the Scott-Knott test ($p \leq 0.05$).

Figure 1 - Phytase activity (mean ± standard deviation) of basidiomycete strains grown in liquid cultivation medium with phytate (5.0 g L^{-1} phytate, 2.0 g L^{-1} yeast extract, 2.0 g L^{-1} $NaNO_3$, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} KCl, 0.1 g L^{-1} $ZnSO_4.7H_2O$, 0.05 g L^{-1} $FeSO_4$) for 21 days. Values indicated by the same letter do not differ statistically by the Scott-Knott test ($p \leq 0.05$).



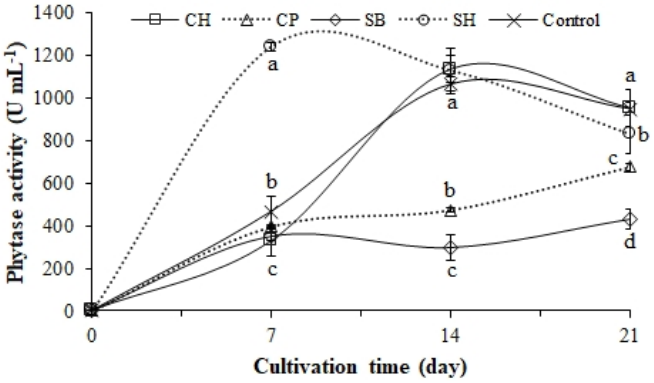
Source: The authors.

The supplementation of the liquid medium with particulate soybean hulls increased ($p \leq 0.05$) phytase activity of *Ganoderma* sp. and anticipated activity peak from the 14th to the 7th cultivation day (Figure 2). Wich represents a 16% increase in the maximum phytase activity and a 2.3-fold increase in productivity (from 76 U mL^{-1} day^{-1} to 177 U mL^{-1} day^{-1}) compared to the medium without agro-industrial byproduct used as control (Figure 2). When particulate coffee husks were added to the liquid medium, the maximum phytase activity did not differ from the control ($p \leq 0.05$). However, the presence of particulate citric pulp or sugarcane bagasse in the liquid medium reduced ($p \leq 0.05$) phytase activity throughout the cultivation period. The maximum phytase activity in the liquid medium with CP and SB was 2.2 and 3.6-fold lower,

respectively, than the maximum activity of the control. We observed a strong negative correlation between the C/N ratio of the particulate agro-industrial byproduct added to the liquid medium and *Ganoderma* sp. phytase activity, particularly on the 14th (r = -0.85) and 21st (r = -0.97) cultivation days. Therefore, this could indicate that the presence of particulate agro-industrial byproducts with a higher C/N ratio disfavors phytase activity (Table 1). The phytase activity of *Ganoderma* sp. growth in particulate SH medium demonstrated good stability at different pH (Figure 3) and temperatures (Figure 4). The residual phytase activity remained above 60% in the pH range (3-9) (Figure 3), except at pH 5, where the residual activity after 120 min was 45% ($t_{1/2}$ = 105 min). The phytase half-life at pH 3 and pH 9 was 162 min and 207.6 min, respectively. Phytase activity at pH 7 remained unchanged throughout the incubation time.

Phytase activity remained above 65% for 120 min when incubated at 30 °C or 50 °C (Figure 4) with a half-life of 192.6 min at both temperatures. After 90 min at 70 °C, phytase activity remained at 60% ($t_{1/2}$ = 122 min), reducing to 45% after 120 min (Figure 4).

Figure 2 - Phytase activity (mean ± standard deviation) of *Ganoderma* sp. grown in liquid cultivation medium (5.0 g L^{-1} phytate, 2.0 g L^{-1} yeast extract, 2.0 g L^{-1} $NaNO_3$, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} KCl, 0.1 g L^{-1} $ZnSO_4.7H_2O$, 0.05 g L^{-1} $FeSO_4$) added with 2.5 g L^{-1} agro-industrial byproducts: coffee husks (CH), citric pulp (CP), sugarcane bagasse (SB), or soybean hulls (SH) or without agro-industrial byproducts addition (control). Values indicated by distinct letters differ statistically by the Scott-Knott test ($p \leq 0.05$).



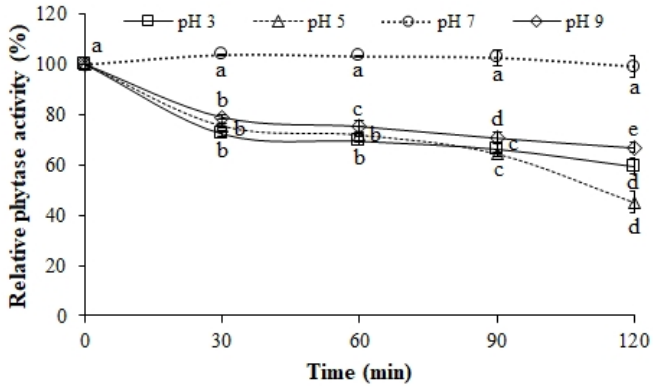
Source: The authors.

DISCUSSION

Ganoderma sp. produced the maximum phytase activity (1108 U mL^{-1}) and productivity (79 U mL^{-1} day^{-1}) in the liquid medium containing phytate as the carbon source. Salmon et al. (2016), who studied *Ganoderma* sp. in SmC with wheat bran cultivation medium (20% wheat bran extract, 2% yeast extract, and 10 mM $CaCl_2$), reported maximum phytase production of 10.7

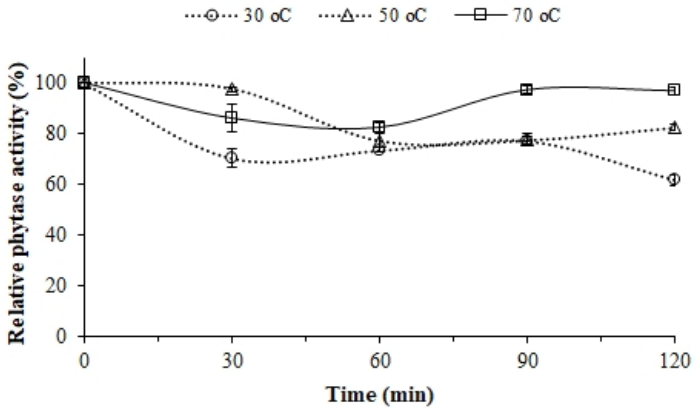
U mL^{-1} after four cultivation days with 2.7 U mL^{-1} day^{-1}, 30-fold lower than observed in our study. Spier et al. (2012) also reported the phytase production by *G. applanatum* and *G. stipitatum*, but in solid substrate cultivation (SSC) with wheat bran. They described that within 72 h, both strains produced 200 U g^{-1} of phytase at 67 U g^{-1} day^{-1}.

Figure 3 - Residual phytase activity (mean ± standard deviation) of *Ganoderma* sp. grown in liquid cultivation medium added with particulate soybean hulls (2.5 g L^{-1}) after incubating at different pH (24 ± 1 °C). Phytase activity in the extract before incubation with buffers was defined as control. Values indicated by distinct letters in each line differ statistically by the Scott-Knott test ($p \leq 0.05$).



Source: The authors.

Figure 4 - Residual phytase activity (mean ± standard deviation) of *Ganoderma* sp. grown in liquid cultivation medium added with particulate soybean hulls (2.5 g L^{-1}) after incubating at different temperatures (pH 5). Phytase activity in the extract before incubation was defined as control. Values indicated by distinct letters in each line differ statistically by the Scott-Knott test ($p \leq 0.05$).



Source: The authors.

The addition of particulate agro-industrial byproducts to the liquid medium containing phytate affected the phytase activity of *Ganoderma* sp. distinctly depending on the byproduct. Pearson correlation index indicated a strong negative correlation between the phytase activity and the C/N ratio of the byproduct added to the cultivation medium. Thus, particulate soybean hulls with a lower C/N ratio favored the phytase activity of *Ganoderma* sp. In contrast, particulate sugarcane bagasse and citric pulp, which have superior C/N ratios, decreased the phytase activity in SmC. Salmon et al. (2016) supplemented the wheat bran-extract cultivation medium with soybean molasses. They reported that the phytase production of *Ganoderma* sp. increased 81%, and this response was attributed to a better C/N ratio provided by soybean molasses. Studies with other basidiomycetes in SmC are less common, but SSC data with Ascomycetes indicate that the C/N ratio of agro-industrial byproducts is an essential parameter for fungal phytase production. Salmon et al. (2012) increased phytase activity of *Schizophyllum commune* in wheat bran SSC added with 5% sucrose by almost 300%. Shahryari et al. (2018) reported maximum phytase activity of *Aspergillus ficuum* in wheat straw cultivation medium after optimizing C/N ratio by adding glucose (0.17 g g^{-1}) and ammonium sulfate (0.07 g g^{-1}). Colla et al. (2022) studied *Lentinus crinitus* and high nitrogen content for sugarcane bagasse pre-hydrolysis with low carbon loss. The authors indicated that the C/N ratio strongly affected the laccase and mushroom production indicated this method for animal feed or microbial succession processes for enzymes, but unfortunately without phytase analyses.

Phytase production is correlated to the initial concentration of inorganic phosphate in the cultivation media that acts as microorganism phytase inhibitor (GULL et al., 2013). Bhavsar et al. (2008) reported that when cultivating *Aspergillus niger* in SmC in an agro-industrial byproduct cultivation medium, the inorganic phosphate content of byproducts was limiting for phytase production due to the occurrence of enzyme production only in cultivation medium with less than 4 mg g^{-1} of phosphate. On the other hand, the presence of phytate in the cultivation medium acts as a phytase activity inducer. The phosphate concentration will inhibit this enzyme when the byproduct phosphate and the phosphate are released by the enzyme action reach critical values (VIVEROS et al., 2000). In our study, the combination of phytate and soybean hulls increased the phytase activity and anticipated the peak of maximum activity seven cultivation days, making productivity twice-fold greater.

Soybean hulls are a potential substrate for phytase production of basidiomycetes, and it is composed of cellulose (29 to 51%), hemicellulose (10 to 20%), pectin (6 to 15%), and lignin (1 to 4%) that favor fungal cultivation and animal feed supplementation (AL LOMAN; JU, 2016). The Brazilian soybean crop of 2020-2021 was more than 138 million tons (CONAB, 2022), and considering that soybean hulls represent from 8 to 10% of the soybean grains and that approximately 50% of the Brazilian soybean is industrially processed, it is estimated that 5-6 million tons of soybean hulls are generated (AL LOMAN; JU, 2016). Furthermore, soybean hulls

contain about 0.2% of phosphorus (STEWART et al., 2012), which did not affect the phytase activity negatively.

Phytases for utilization in the feed industry must be capable of acting at different pH and temperatures. Livestock and poultry have low pH in the stomach. Therefore, acidophilic phytases, stable in acid pH, which act at low pH and resist this acid denaturation, are of particular interest (CORRÊA; ARAÚJO, 2020). On the other hand, many species used in aquaculture have neuter digestive pH, demanding functional phytases that are resistant in slightly acid or close to neutrality pH (CAO et al., 2007), besides being resistant to typical temperatures of aquaculture environment (ranging from 0 °C to 25 °C). We evaluated the phytase stability of *Ganoderma* sp. grown in a cultivation medium with particulate soybean hulls at different pH and temperatures. Partially purified phytase of *Agaricus bisporus* kept stable from pH 5 to 8 after 60 min of incubation (COLLOPY; ROYSE, 2004). Phytases extracted from *Flammulina velutipes* (ZHU et al, 2011), *Volvariella volvacea* (XU et al., 2012), and *Lentinula edodes* (ZHANG et al., 2013) basidiocarps kept residual activity over 70% after incubation at pH ranging from 3 to 9. Our results demonstrated that phytase presented stability in neuter pH, keeping its activity unchanged for 120 minutes and residual activity over 65% at all pH levels until 90 minutes of incubation. These results are following the literature that describes phytases of functional and stable basidiomycetes in broad pH ranges. Thus, this characteristic indicates an advantage for *Ganoderma* sp. Phytase to develop applications such as feed additives, mainly in aquaculture. Phytase of *A. flavus* kept more than 75% of its activity in pH varying from 6 to 9, but 30 minutes incubation in pH < 6 and pH > 9 significantly reduced the phytase activity (GAIND; SINGH, 2015).

Phytase thermostability is considered essential, and it is a criterion utilized to choose phytases for different applications. Thermostable phytases from various sources show distinct levels of stability to heat and, given the high temperatures employed in the pelleting process of animal feed, its thermal stability is necessary (CORRÊA; ARAÚJO, 2020). Zhang et al. (2013) reported that *L. edodes* phytase, after one-hour incubation, kept approximately 90% of its activity at 45 ^{o}C and 80% at 60 ^{o}C. Similar results to *Ganoderma* sp. phytase produced in our study that, after one hour, kept 85% of the enzymatic activity at 30 ^{o}C, 70% at 50 ^{o}C, and 60% at 70 ^{o}C. In our study, *Ganoderma* sp. phytase showed greater stability than *S. commune* phytase (SALMON et al., 2012), which, after 30-min incubation, kept less than 60% of the activity at 60 ^{o}C, and only 37% at 70 ^{o}C. A purified phytase of *A. bisporus* maintained 100% of its activity at 37 ^{o}C, but it was utterly inactive after 120 min at 60 ^{o}C (COLLOPY; ROYSE, 2004). The comparison data of basidiomycete phytase thermostability indicate significant variability among different species and suggest that further studies on basidiomycete phytases are necessary to reveal the activity amplitude of these enzymes. However, the characteristics of thermal stability and neutral-pH stability of *Ganoderma* sp. phytase produced by SmC with particulate soybean hulls showed potential for application in the animal feed industry, mainly in aquaculture. Moreover, the

cultivation utilizing agro-industrial byproducts allows enzyme production at lower costs, aggregating value to the byproducts and generating knowledge that may contribute to the production of this enzyme on an industrial scale.

CONCLUSION

Polyporales strains such as *Ganoderma* sp., *Lentinus berteroi*, *Lentinus crinitus*, and *Pycnoporus sanguineus* produce high phytase activity in SmC containing phytate as the only source of carbon and phosphorus. The addition of particulate soybean hulls to the liquid medium with phytate increases the activity and productivity of *Ganoderma* sp. phytase. On the seventh cultivation day, the highest phytase activity (1130 U mL^{-1}) and productivity (177 U mL^{-1} day^{-1}) are produced. *Ganoderma* sp. phytase is stable in pH ranging from 3 to 9 and keeps 100% enzymatic activity at pH 7 after 120 minutes, and more than 60% enzymatic activity from 30 ^{o}C to 70 ^{o}C for 120 min. Our study showed that the thermostability characteristics and neutral-pH stability of *Ganoderma* sp. phytase produced in SmC with particulate soybean hulls are an excellent alternative for the application in the animal feed industry, mainly in aquaculture. Further optimization studies of production conditions of this enzyme by *Ganoderma* sp. may support the production in the cultivation medium with the addition of particulate soybean hulls, aggregating value to the agro-industrial productive chain.

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